United States Patent [19]

Eisinger et al.

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[45] **Date of Patent: Jul. 24, 1990**

[54] **LATERAL FLOW, NON-BIBULOUS MEMBRANE ASSAY PROTOCOLS**

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[21] Appl. No.: **230,642**

102(e) [22] Filed: **Aug. 10, 1988**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 57,273, Jun. 1, 1987, abandoned, and a continuation-in-part of Ser. No. 57,271, Jun. 1, 1987, abandoned.

[51] **Int. Cl.**[5] **G01N 33/53**

[52] **U.S. Cl.** **435/7**; 435/805; 435/810; 436/512; 436/514; 436/518; 436/520; 436/523; 436/531; 436/535; 436/807; 436/808; 436/810; 422/55; 422/56; 422/57; 422/58; 422/101

[58] **Field of Search** 422/55–61, 422/70, 101, 102; 424/11; 435/7, 5, 805, 810; 436/514–520, 512, 523, 531, 535, 807, 808, 810; 210/431

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[57] **ABSTRACT**

A method and apparatus for conducting specific binding pair assays, such as immunoassays, is described. A porous membrane capable of non-bibulous lateral flow is used as assay substrate; a member of the binding pair is affixed in an indicator zone defined in the substrate. The sample is applied at a position distant from the indicator zone and permitted to flow laterally through the zone; any analyte in the sample is complexed by the affixed specific binding member, and detected. A novel method of detection employs entrapment of observable particle in the complex. Blood is a particularly preferred sample as the red blood cells can be used as the observable particles for detection of the complex.

46 Claims, 4 Drawing Sheets

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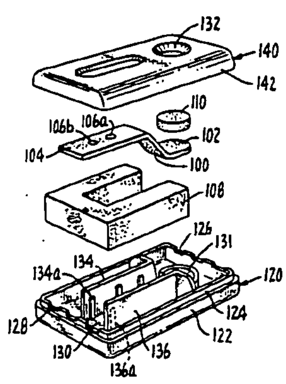

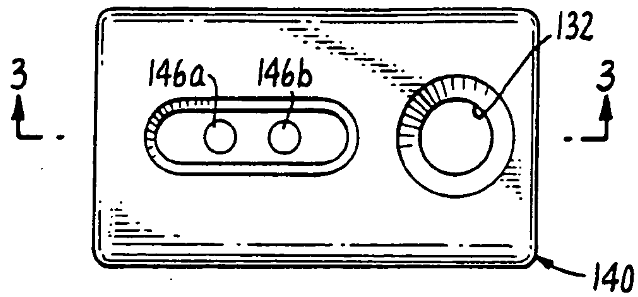

FIG. 1.

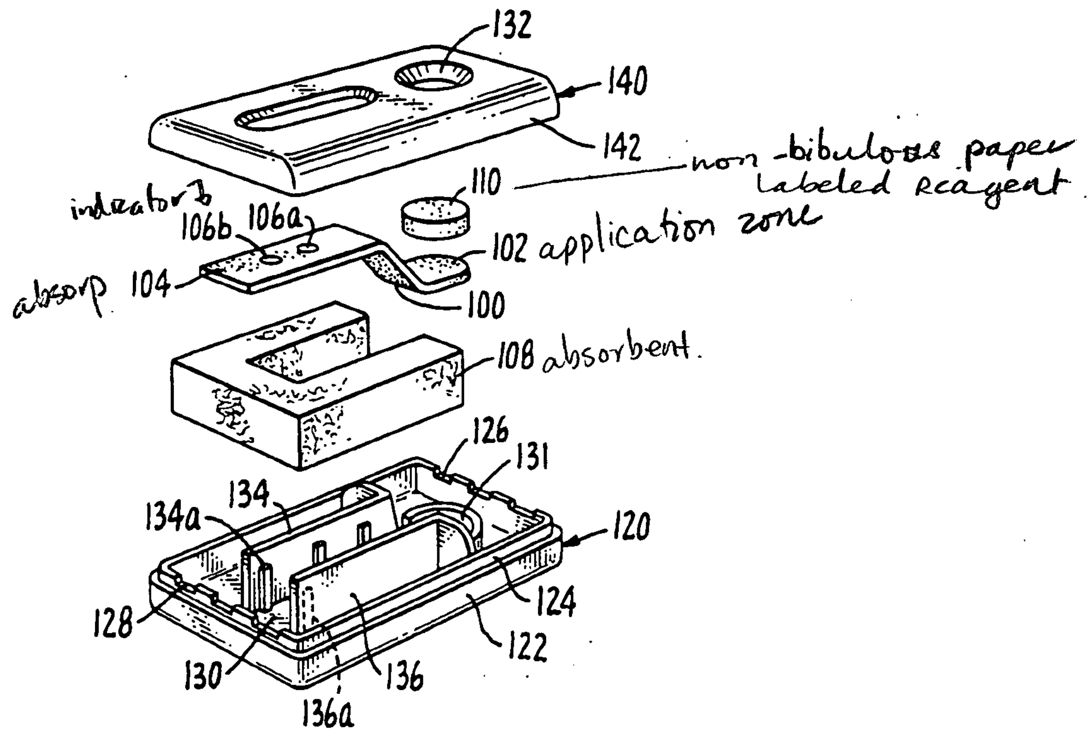

FIG. 2.

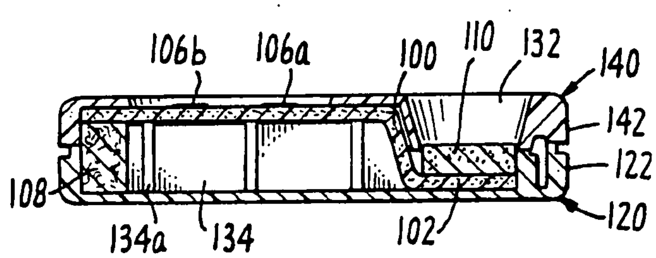

FIG. 3

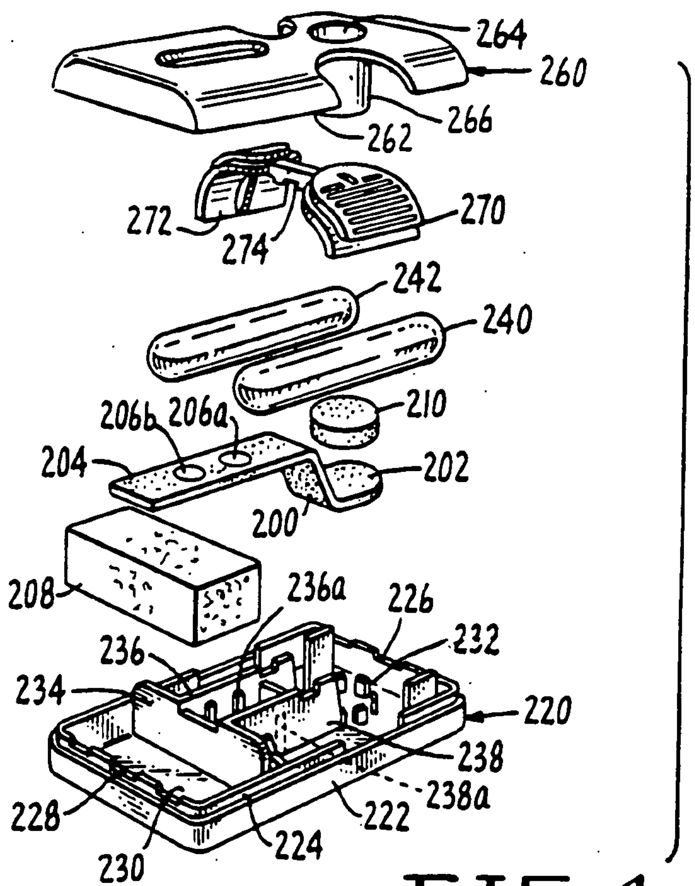
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FIG.4.

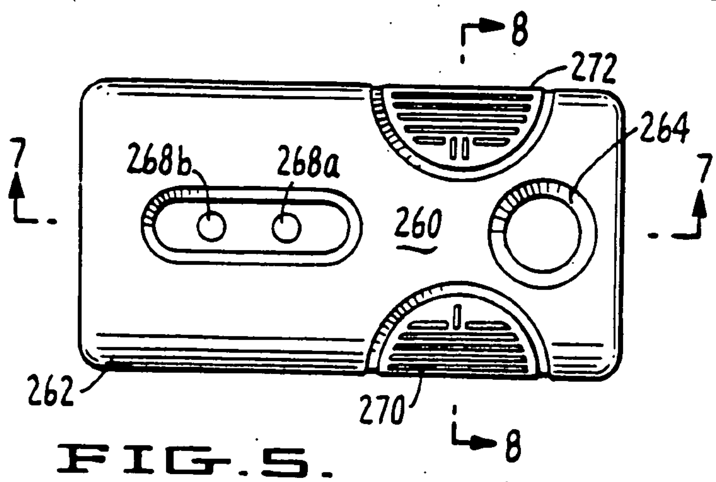
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FIG.5.

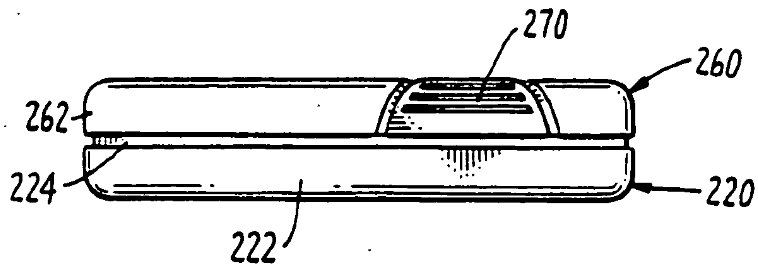

FIG. 6.

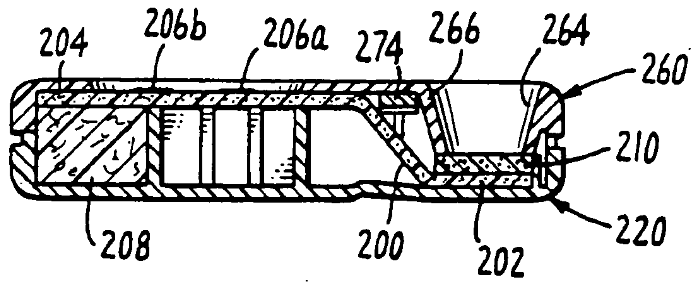

FIG. 7.

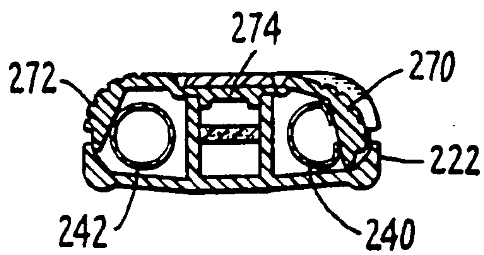

FIG. 8.

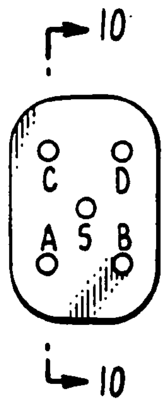

FIG. 9

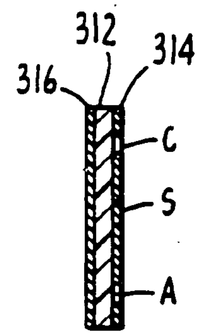

FIG. 10.

LATERAL FLOW, NON-BIBULOUS MEMBRANE ASSAY PROTOCOLS

This is a continuation-in-part of copending application Ser. Nos. 057,273 and 057,271, both filed June 1, 1987, and now abandoned.

FIELD OF THE INVENTION

This invention relates to immunological and related assay methods and apparatus, especially to those for blood testing.

BACKGROUND OF THE INVENTION

The technology of specific binding diagnostic reactions and reagents generally has developed rapidly in the past two decades, and continues to develop at a rapid pace. Radiommunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs), for example, have become widely known and are described in numerous tests, treatises, scientific papers and patents. ELISAs have become commonplace and achieved great importance in medicine and in scientific research since the pioneering work begun by Engvall, E., and Perlmann, P., et al, *Immunochem* (1971) 8:871-874; and the work of Schuurs and coworkers; see, e.g., Van Weemen, *FEBS Letters* (1971) 15:232-236, and several U.S. Patents naming Schuurs et al as inventors; see, e.g., U.S. Pat. Nos. Re. 31,006, 3,654,090, 3,839,153, 3,850,752, 3,862,302, 3,862,928, 3,879,262, and 4,016,043. Monoclonal antibodies in enzyme immunoassays are well known; see, e.g., the work of Herzenberg and of Engvall, and others and the later work of David et al, U.S. Pat. No. 4,376,110. DNA probes and biochemical and biological probes generally exhibit the ability to bind specifically and are of great current interest as specific binding pairs.

ENZYME IMMUNOASSAY, Ishikawa, M. D., Tadashi, Kawai, and Kiyoshi, Miyai, eds, IGAKU-SHOIN, New York 1981, describes in considerable fundamental detail the principles and practices involved in enzyme immunoassays. Reference is also made to other texts and treatises in the field, such as *IMMUNOCHEMICAL METHODS IN THE BIOLOGICAL SCIENCES: ENZYMES AND PROTEINS,* Mayer, R. J., and Walker, J. H., Academic Press, New York 1980; *QUANTITATIVE ENZYME IMMUNOASSAY,* Engvall, E., and Pesce, A. J., Blackwell Scientific Publications, London (Scandinavian Journal of Immunology, 1978); *THE ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) A guide with abstracts of microplate applications,* Voller, A., Bidwell, D. E., and Bartlett, A., Dynatech Laboratories, Inc., 1979; and the references cited therein for a comprehensive disclosure of the principles and usual practices involved in enzyme immunoassay.

Various approaches have been described for carrying out enzyme immunoassays. The early ELISAs were what is commonly called a "competitive" assay in which the enzyme labeled antigen or antibody competed with the antigen or antibody to be determined for a reaction site on a bead, pad or surface to which one member of an immunologically coupling pair was attached. Later, the "sandwich" assay became popular. In the sandwich assay, the antibody or antigen to be determined was "sandwiched" by an immunochemical reaction between a solid surface treated with an immunological species reactive with the species to be determined

and the same or a different reactive immunological species which has been coupled to an enzyme label. The principles of these types of ELISAs are discussed by Belanger, L., *Scand J Immunol,* (1978) 8:Suppl. 7, 33-41; (Chapter 4 in *QUANTITATIVE ENZYME IMMUNOASSAY,* supra).

Many forms of solid supports to which one member of an immunochemical couple, e.g., antigen-antibody or hapten-antibody couple, have been disclosed. A common early form of solid support was a plate, tube or bead of polystyrene which was well-known from radioimmunoassay (RIA) work to bind certain immunological species. Filter paper, glass, various plastics (chemical polymers), and other solid support surfaces have been used for many years. Examples of such a system which used antibody (or antigen) coated polystyrene beads are described by Bohn et al, in U.S. Pat. No. 4,424,279, Jan. 3, 1984; and U.S. Pat. No. 4,458,020, July 3, 1984, in which the coated beads are utilized in unique configurations.

Several disclosures are directed to assays which employ passage of the sample to be tested through a solid membrane or support.

Tom et al, U.S. Pat. No. 4,366,241, disclose an apparatus for an immunoassay which includes a multiple layered construction in which the sample solution flows into an enclosure through an immunoabsorbing disk which has antibody or antigen bound to it. The solution flows from the disk through a membrane spacer which is in contact with the disk and into a bibulous strip of cellulose or paper which extends through the enclosure to a level above the sample into which the apparatus is inserted during use.

U.S. Pat. No. 4,632,901, to Valkirs et al, discloses a device and method for immunoassays in which the sample flows through the thickness of a membrane to an absorbent mass. Antibody is bound to less than the total surface of the membrane and binds antigen in the antibody coated area. Conventional ELISA techniques are used to detect the sample bound to the supported antibody.

Various configurations for self-contained assay systems have also been described; for example, Deutsch, A., and Platt, H. A., U.S. Pat. No. 4,522,923, describe a device which comprises a container, at least two water-soluble barriers, subdividing the container into at least three superimposed chambers, and different biologically active substances in each chamber. Upon introduction of an aqueous biological sample to be tested into the topmost chamber, the sample successively mixes with the contents of the chambers, the contact time in each chamber being a function of the water solubility of the barriers. The system is designed to give a color reading in the final chamber. This provides a method for conducting immunochemical reactions in a self-contained sealed unit that requires only the addition of an unknown sample and water, and thus provides an assay system that is safe and accurate even when used by an individual who is not technically trained. Specifically at least one chamber contains an antigen, antibody, or an enzyme, or their conjugates. Preferably the antibody is directed against human chorionic gonadotropin hormone. Specifically the substances in the chambers represent color-change immunochemical reactions, e.g., home testing of blood or urine for pregnancy.

Barnett, B., W08606488, describes a diagnostic test kit which has a central well for receiving a sample to be analyzed. Several reservoirs holding predetermined

quantities of reagents are located in a block which surrounds the sample well and are connected to it via bores. Initially the reagents are retained in the respective reservoirs by membranes but the contents of a reservoir can be discharged by rupturing the membrane. The reservoirs are formed by resilient domes which are depressed manually to rupture the membrane and serve to transfer the reagent to the sample well. The test kit is used for screening, chemical or clinical analysis of blood, urine, swimming pool water, drinking water or soil. The test kit reduces the chance of human error in the sequential addition of reagents to a sample.

Graham, H. A., Olekna, D. J., Hawk, J. B., and Kebles, D. B., EP0022669, describe a test in which red blood cells are rapidly tested for the presence of antigens O, C, c, E, e or K by mixing them with an antibody reagent (A) and, without incubation, examining them for agglutination. (A) comprises reduced S-alkylated IgG antibody against the appropriate antigen which at least meets FDA standards for potency and specificity.

Deutsch, M. E., and Mead, L. W., U.S. Pat. Nos. 4,094,647, 4,235,601 and 4,361,537, describe a test strip for determining a characteristic of a sample comprises a length of material capable of transporting a developing liquid by capillarity and having zones between its ends for receiving the sample and holding reagents. The strip is used for performing binding assays, particularly those in which a radioisotope is used as a label, such as radioimmunoassays. Minute sample sizes may be used. The strip is capable of application to analytical methods having sensitivities below 0.1 mg/ml.

Friedenberg, R. M., FR 2537724, describes a dry indicator apparatus for drugs-of-abuse testing of physiological liquid test solutions. A non-bibulous capillary flow membrane vehicle matrix is impregnated with dry chemical colorants. When these are placed in contact with the test solution the colored reagent indicates the type of drug present even in low concentrations. The test is a qualitative and quantitative indicator for the presence of abused drugs, such as barbiturates, amphetamines, methadone, morphine, cocaine, codeine, dilaulid and tranquilizers of the diazepam type. The physiological fluids tested include urine, whole blood, plasma, sweat and tears.

Lipp, V., and Buck, R. L., EP 0206779, describe an apparatus for detecting antinuclear antibody in a biological sample comprising a solid support having adhered nuclei isolated from eucaryotic cells. Preferably underlying the nuclei on the solid support is a coating, e.g., of nuclear antigens, which is unreactive with antibodies to non-nuclear antigens and which, like nuclei serves to bind antinuclear antibodies in the sample. The apparatus permits the screening of human serum for the presence of antinuclear antibodies in a system featuring speed, simplicity, sensitivity and capacity for automation. Medical disorders characterized by the presence of antinuclear antibodies include systemic lupus erythematosus, mixed connective tissue disease, Sjogren's syndrome and scleroderma.

Deutsch, A., Sheets, E. J., and Rhodes, J., EP 0189925, describe a kit which comprises (a) a vessel, (b) a capillary-active wick extending from the interior of the vessel so as to wick a liquid out of the interior of the vessel, a portion of the wick carrying an immobilized immunological component selected from (i) antigen and (ii) antibody, (c) a first reagent comprising an enzyme conjugated to an immunological component selected from (i) antibody and (ii) antigen specific to (i) or (ii) respectively of (b) and (d) a substrate for the enzyme. In place of the enzyme and substrate a fluorescent label may be used. Antigens which can be tested for include dilantin, testosterone and progesterone. If the sample contains the antigen, it will combine with the antibody-enzyme while moving along the wick so that when this mixture subsequently wicks through the antigen-wick, there will be no free antibody-enzyme to bind to the antigen on the wick so it will pass out of the wick.

Friedenberg, WPO Int. Pub. No. WO 84/02397, also describes an immunoassay in which the reactions occur in the liquid phase as the sample moves through a paper support, the rate of movement being one parameter used in identifying constituents.

Campbell, U.S. Pat. No. 3,893,808, describes a strip of filter paper treated in bands with a chemical reagent, iodine, into which a sample of gasoline suspected of containing lead is wicked from one end and a developing reagent, dithizone, is wicked into the pretreated bands.

Alberty et al, U.S. Pat. No. 3,895,914, describe another chemically treated test strip in which chemical reagents are applied in bands or zones on a strip for detecting barbituric acid.

While the prior art teaches the use of wicking bibulous materials as carriers for specific binding reagents, these apparatus and methods rely principally upon the ability of the carrier to imbibe the liquid and often to enter into the reaction. The use of bibulous materials is of great value in some methods, but presents serious limitations as well, in reduced sensitivity and in the nature of the reagents and analytes which may be used or determined. The present invention utilizes a non-bibulous material in which the liquid flow is isotropic and flows laterally in the material by capillary action, thus presenting a system in which the solid membrane provides a vessel for the liquid but does not imbibe or otherwise enter into or interfere with the specific binding reactions.

DISCLOSURE OF THE INVENTION

The invention provides a method and apparatus for determining the presence or absence or the amount of analyte using a specific binding assay. The apparatus comprises a non-bibulous lateral flow membrane which has on its surface a sample application zone to receive a liquid sample, and, at a lateral distance from the application zone on the surface, at least one indicator zone. In the indicator zone is affixed a member of a binding pair; the sample contains an analyte which is its complementary binding member or an analyte which can be derivatized so as to bind the fixed member. In one convenient configuration the membrane is bound to two substantially fluid-impervious sheets, one on either side, with openings on one side or both sides to provide definition to the application and indicator zones.

The lateral flow achieved in the method of the invention is the result of the properties of the non-bibulous lateral flow membrane. The membrane has a much smaller thickness than surface dimension and is hydrophilic enough to be wetted and thus permit aqueous solutions and materials to exhibit lateral flow freely, and preferably isotropically, at substantially the same rates for various components of a sample.

Thus, in one aspect, the invention is directed to an apparatus for assaying an analyte in a sample to be tested by a specific binding reaction which apparatus comprises a non-bibulous lateral flow membrane, said

membrane having at least one sample application zone and at least one indicator zone, said zones laterally separated, and wherein in the indicator zone is affixed a member of a binding pair.

The apparatus may further comprise one or more breakable containers of buffers or reagents positioned in a holder adjacent the membrane. These can be broken by a means which may also be included in the holder, to provide needed developing reagents or wash solutions to the indicator zone when the container is broken. Another aspect of the invention comprises such designs.

multiple analytes

The membrane may include more than one indicator zone, along with control and reference zones. Multiple indicator zones may be designed to detect different analytes or for quantifying the amount of analyte. Multiple indicator zones may be in any spaced relationship to the application zone, since the membrane itself does not provide a barrier to sample flow.

In the method of the invention, the sample, which contains an analyte which is, or which can be derivatized to include, a first member of a binding pair, is applied to the application zone and allowed to be transported laterally through the membrane to an indicator zone, where there is, affixed to the membrane, the other, second, member of the binding pair. The first member binds, in the indicator zone, to the second member, and the resulting bound complex is detected. Detection may use any of a variety of labels and/or markers, e.g., enzymes, radioisotopes, liposomes, fluorescent tags, polymer dyes, or colored particles, etc., and detection is by means of, for example, direct visual observation, by developing a color, by radioactive isotope counting, by fluorescence measurement, or by any of many other techniques by which the presence or absence of a chemical or biochemical species may be detected directly or indirectly.

In one preferred embodiment, and an additional aspect of the invention, visible particles in, added to, or applied before or after, the sample are used for detection by being trapped in the indicator zone by the binding pair complex. If the analyte itself provides a visible particle, for example, in the case of analyte being an antigen present on red blood cells, which cells can be seen directly, no separate detection means is needed. If the visible particles reside in the sample, e.g., the red blood cells in whole blood will remain in the indicator zone after washing when the binding complex is formed.

Thus, in another aspect, the invention is directed to a method to detect the formation of a complex between a first binding reaction pair member from a sample and the second member of the pair affixed in an indicator zone, which method comprises supplying, along with or after the sample, detectable particulates which are entrapped by the complex and thus detected in the indicator zone. The particles may occur naturally in the sample as is the case for the red blood cells in whole blood. In other instances, the particles may be added artificially.

however, it is clearly obvious to one of ordinary skill in the art

In another aspect, the invention is directed to a method of blood typing using the membrane described. A blood sample is applied to the application zone and permitted to flow through the membrane to one or more indicator zones, each of which contains a blood typing reagent, such as an antibody to group A antigen, to group B antigen or to Rh factor. The blood sample is then washed through the membrane so that the red blood cells remain visible only in the indicator zones which contain the specific binding member of the pair pertinent to an antigen present in the sample. Thus, for example, type A blood will be visible in an indicator zone containing anti-A; blood lacking this antigen will be removed from this zone, which will appear clear.

In other aspects, the invention relates to specific configurations of the application and indicator zones on the membrane.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a top plan view of the holder in a preferred embodiment of the invention.

FIG. 2 is an exploded perspective view of the holder of FIG. 1 showing the components thereof and the relationship of the membrane and absorbent components contained herein.

FIG. 3 is a side cross-sectional view of the apparatus of FIG. 1, take along lines 3—3 of FIG. 1 in the direction of the arrows.

FIG. 4 is an exploded view of another preferred embodiment of the apparatus of this invention which includes reagents as well as the membrane and absorbent body.

FIG. 5 is a top plan view of the apparatus of FIG. 4.

FIG. 6 is a side elevational view of the apparatus of FIG. 5.

FIG. 7 is a side cross-sectional view of the apparatus of FIG. 5 taken along lines 7—7 of FIG. 5 in the direction of the arrows.

FIG. 8 is a transverse cross-sectional view of the apparatus of FIG. 5 taken along lines 8—8 of FIG. 8 in the direction of the arrows showing the breaking mechanism of the invention.

FIG. 9 is a plan view of an alternative, simplified form of an apparatus suitable for use in this invention, especially for blood typing.

FIG. 10 is a cross-sectional view of the apparatus of FIG. 9.

MODES OF CARRYING OUT THE INVENTION

General Description

An essential feature of the invention is the employment of a membrane capable of non-bibulous lateral flow. By "non-bibulous" lateral flow is meant liquid flow in which all of the dissolved or dispersed components of the liquid are carried at substantially equal rates and with relatively unimpaired flow laterally through the membrane, as opposed to preferential retention of one or more components as would occur, for example, in materials capable of adsorbing or "imbibing" one or more components. "Bibulous" materials include paper, nitrocellulose, nylon and the like, which have the capability to effect a chromatographic separation of the contained materials.

An example of the membrane material in which capillary, non-bibulous lateral flow occurs is the high density or ultra high molecular weight polyethylene sheet material manufactured by Porex Technologies Corp. of Fairburn, Georgia, USA. This membrane has an open pore structure with a typical density, at 40% void volume, of 0.57 gm/cc and an average pore diameter of 1 to 250 micrometers, the average generally being from 3 to 100 micrometers. The optimum pore diameter for the membrane for use in the invention is about 10 to about 50 μm. The membranes are from a few mils (0.001 in) to several mils in thickness, typically in the range of from 5 or 10 mils and up to 200 mils. The membrane may be

Same pore size as macroporous

backed with a generally water impervious layer, or may be totally free standing. While membranes made of polyethylene have been found to be highly satisfactory, lateral flow, non-bibulous membranes formed of other olefin or other thermoplastic materials, e.g., polyvinyl chloride, polyvinyl acetate, copolymers of vinyl acetate and vinyl chloride, polyamide, polycarbonate, polystyrene, etc., can be used. Membranes formed by the classical phase inversion process may also be used.

Thus, the membranes, in general, will have a pore size of about 3–100 μm, preferably about 10–50 μm; will be constructed of an inert material; and will be less than 200 mils in thickness. They are characterized by nonbibulous lateral flow. Isotropic flow is preferred. While applicants believe this lateral flow to be caused, at least in part, by capillary action, they are not bound by any particular theory to explain the characteristic nature of this non-chromatographic flow.

In the various apparatus and method embodiments of the invention, the lateral flow non-bibulous membrane will contain an application zone and at least one indicator zone, wherein the indicator zone has affixed to it a member of a binding pair. The membrane may be in any desired shape and the application and indicator zone may have any desired configuration as is convenient for use alone or in a particular apparatus.

To the indicator zone in the apparatus and method of the invention is affixed one of the members of a binding pair, which is responsible for the capture of its complementary member. By "affixed" in this context is meant retained in the indicator zone throughout the assay procedure—this can be accomplished by covalent bonding or, more commonly, by adsorption, e.g., by drying. Depending on the nature of the material comprising the membrane, derivatization to permit covalent bonding for example, using glutaraldehyde or a carbodiimide, can be employed.

Most binding pairs employed in the invention are "specific", e.g., antigen-antibody pairs, and other specific coupling pairs such as antibody-hapten, antibody-cell, antibody-cell fragment, RNA and DNA probes, receptor-receptor ligand, enzyme-substrate, enzyme-inhibitor and other pairs in which a specific binding reaction occurs. However, in some instances the specificity of the assay may be conferred in other ways, such as by a labeling reagent. The requirement for the affixed member is that it must bind the analyte or its derivative. Thus, one of the members of the coupling pair is affixed physically, chemically, biologically or otherwise to the non-bibulous lateral flow membrane indicator zone to bind the other member of the pair.

The affixed member of the pair may bind directly to the analyte, or may bind to a derivative thereof. By "derivative" is meant any substance whose concentration in the sample is directly proportional to analyte. For example, the derivative may be a conjugate of the analyte with an additional component which, in turn, binds to the affixed member, or with an additional component which serves merely to label the analyte, but not interfere with the analyte's ability to bind to the affixed member. In another illustration, the derivative might be a reaction product formed in stoichiometric relationship to analyte in a reaction, wherein the reaction product binds to the affixed member. Thus, "derivative" is a substance quantitatively related to analyte concentration.

It is not necessary that the binding pair member be bound directly, chemically or biologically to the membrane. The binding pair member may be attached to another material wherein said material is physically entrapped in the indicator zone or otherwise affixed in the indicator zone by any physical, chemical or biochemical means. For example, specific binding members can be attached covalently or passively to beads or the like and the beads then affixed on the membrane.

The method of the invention is conducted by applying a liquid sample to the application zone at the surface of the membrane in sufficient quantity to permit the sample to flow through at least one, or through at least as many indicator zones as desired. Control and blank zones may also be defined to receive the sample flow. Flow will occur laterally through the membrane due to its intrinsic properties; while applicants believe this is due to capillary action, there is no intent to be bound to any particular theory or explanation. If, along the direction of flow, the membrane terminates at the indicator zone, the liquid may flow out of the membrane; if there is additional surface beyond the indicator zone in the direction of flow, this surface will act as an "absorbent" zone, and further encourage the flow of liquid. A bibulous or nonbibulous material may also be placed in contact with the membrane to act as additional absorbent.

In the method, then, the sample flows from the application zone through the indicator zone and, if applicable, into the absorbent zone. The presence of analyte in the sample will cause a detectable reaction in the indicator zone.

The experimental design protocol or "chemistry" of the assays of the invention can be varied as is generally known for assays based on specific binding. Most of the protocols adapted to other physical formats of immunoassay can be used in the apparatus of the invention, where the indicator zone fills the role of the binding member bound to solid support. For example:

(1) Analyte in the sample binds specifically to the member affixed to the indicator zone; a label is then added to detect the bound analyte; i.e., in a sandwich immunoassay;

(2) The sample is spiked with a labeled form of analyte and the labeled form bound to the member affixed in the indicator zone is detected—i.e., a competition assay;

(3) Analyte carries with it the means of its own detection—the most notable example being analyte conjugated to an observable particle. Also, the analyte may first be reacted with label, for example, with an antibody conjugated to enzyme. The label-bearing analyte can then be bound specifically to the affixed member. In an alternative, a labeled analyte may be bound to a specific counterpart which is the complement to the fixed member. For example, red blood cell-borne analyte may be reacted with murine anti-antigen to form a complex, which is then bound to rabbit antimurine Ig affixed in the indicator zone;

(4) Detectable particles may be used to detect an unlabeled complex of analyte with binding pair member in the indicator zone.

Typical Analytes

The analyte may be insoluble or attached to insoluble supports or may be soluble.

Typical cell-bound or solid supported analytes include, e.g.,

Tissue-Specific Cell Surface Markers: Separation of cell populations based on these markers has been per-

formed using lectins (Reisner and Sharon, *Trends in Biochem Sci* (TIBS) 29, 1980), blood leukocyte surface glycoproteins (Gahmberg and Anderssen, *NYAS* (1978) 312, in *Fibroblast Surface Proteins* eds. Vahery, Ruslahti and Mosher), estrogen steroid receptors (Thompson, *Cancer Treatment Reports* (1978) 63(2) 180, erythrocyte insulin receptors (Bhathena et al, *Horm Metab Res* (1981) 13:179), or multiple markers as in the case of lymphocytes. Further separation of subpopulations is possible based on markers identified with specific cell functions as in the case of the T lymphocytes (Reinberg and Schlossman, *N Eng J Med* (1980) 303:1153).

Tissue-Shared Cell Surface Markers: Some cell surface markers are present on multiple cell types. An example of these are the Major Histocompatibility Complex Human Lymphocyte Antigen (HLA) system, LETS protein, p glycoprotein (Kartner et al, *Science* (1983) 221:1285) and transferrin receptors (Omary et al, *Nature* (London) (1980) 286:888).

Viral-Associated Cell Surface Markers: Cell membrane antigens can also result from viral infection. The mumps H—N glycoprotein detectable by RIA, immunofluorescence and hemagglutination inhibition represents a viral marker on infected cells (Sever et al, *Infect & Immun* (1982) 35(1):179). Similarly, markers resulting from Herpes Simplex 1 and 2 infection are recognizable on the host cell surface by immunofluorescence (Stewart and Herrmann, "Herpes Simplex Virus" in *Manual of Clinical Immunology*, 2nd edition, edited by N. R. Rose and H. Friedman, American Society for Microbiology, Washington, D.C., 1980).

Tumor-Specific Cell Surface Markers: Neoplastic and oncogenic transformation results in the alteration of the cell phenotype as expressed in cell surface proteins. These can be observed as variations in the presence of cell surface antigens normally expressed on the cell surface, appearance of "altered self antigens," appearance of embryonic cell surface antigens and the presence of tumor specific molecules. Felsted et al (*Canc Res* (1983) 43:2754) have described cell membrane changes during the differentiation of promyelocytic leukemia cells. Neoplastic transformation induced changes in cell phenotype are presented in a review by Poste (in *Cancer Invasion and Metastasis: Biologic Mechanisms and Therapy* edited by S. B. Day et al, Raven Press, New York, 1977). Similar review articles describe phenotypes of leukemic cells (Greaves et al in *Proc of International Symposium on Human Lymphocyte Differentiation: Its Application to Cancer*, edited by Seron and Rosenfeld, North Holland Publishing, Amsterdam, 1978), B Lymphocytes (Thorsky et al, IBID), and Acute Lymphocytic Leukemia Cells (Greaves et al, *Science* 234, 1986).

The identification of tumor specific antigens or markers and their association with tumors of specific tissue types permits clearer diagnosis and subsequent monitoring during therapy. A number of tumor surface proteins have been identified. Several examples include: a mutated rat gene p21 tumor lymphocyte protein (Bos et al, *Nature* (London) (1985) 315:726, and Clark et al, *PNAS* (USA) (1985) 82:5280); an Acute Lymphocyte Leukemia (ALL) Associated antigen GP 100 Ph1 (Greaves et al, *Blood* (1983) 61:628); Human T cell Leukemia Associated Antigen (HTLA) (Seon et al, *J of Immunol* (1981) 127(6):2580); a Human Lung Tumor Associated Antigen (Braatz et al, *J Nat Cancer Inst* (1978) 61(4):1035), an estrogen 24,000 MW Human breast cancer marker (Adams et al, *Cancer Res* (1983) 43:4297); a Human Leiomyosarcoma antigen (Deng et al, *Lancet*, Feb. 21, 1981, p. 403); and a Human Mammary carcinoma antigen (Schlom et al, *PNAS* (1980) 77 (11):6841).

Further concerning tumor markers, the concept of "altered self antigens" proposed by Edelman, *Science* (1976) 197:218 describes the presence of modified cell surface antigens normally indigenous to a cell type which are altered due to neoplastic transformation. These aberrant cells are viewed by the immune surveillance system as abnormal and they are capable of eliciting an immune response (Burnet, *Brit Med J* (1957) 1:179, and *Nature* (1970) 226:123). The reappearance of embryonic antigens have also been observed following the neoplastic transformation of cells. Carcinoembryonic antigen (CEA), Fetal Embryonic antigen (FEA) and Tumor Specific Transplantation Antigens (TSTA) have been useful in the serodiagnostic detection of carcinomas and sarcomas (Mitchison, "Immune Surveillance" in *B and T Cells in Immune Recognition* edited by F. Loors and G. E. Roelants, Wiley and Sons, New York, 1977).

A preferred analyte is the set of antigens found on red blood cells, wherein the cells themselves can serve as markers.

In addition to surface markers, soluble analytes can also be detected and measured. Typical soluble analytes include: hormones, enzymes, lipoproteins, viral antigens, immunoglobulins, lymphokines, cytokines, drugs, soluble cancer antigens, bacterial antigens and the like. These analytes include various proteins such as protamines, histones, phosphorylated proteins, nucleoproteins, and so forth such as, for example, transcortin, erythropoeitin, transferrin, various globulins, thyroxin-binding globulin, the immunoglobulins of various subclasses A, G, D, E, and M, various complement factors, blood clotting factors such as fibrinogen, Factor VIII, tissue thromboplastin, and thrombin.

Also included are hormones such as insulin, glucagon, relaxin, thyrotropin, somatotropin, gonadotropin, follicle-stimulating hormone, gastrin, bradykinin, vasopressin, and various releasing factors. A wide range of antigenic polysaccharides can also be determined such as those from *Neisseria gonorrheae*, *Pasteurella pestis*, *Shigella dysentereae*, and certain fungi such as Mycosporum and Aspergillus. An extensive list of soluble analytes determinable in the method of the invention is found in U.S. Pat. No. 3,996,345, which is incorporated herein by reference.

Apparatus Designs

A number of designs for the apparatus comprising the membrane can be used for illustration. The membrane sheet may be of any shape and of almost any size which may conveniently be handled. In a preferred embodiment, a "small" size may be used so that the sheet is suitable for being held in the hand of a user without tearing or breaking, and is generally limited to about the size of an adult human hand, though size per se is not critical. The sheet may be, and preferably is, mounted in a suitable holder provided with means for applying the sample and any necessary reagents to the sheet.

In one illustrative design, as shown in FIGS. 1–3, the lateral flow, non-bibulous membrane **100** is an elongate strip. The membrane, has an application zone **102** which is adapted to receive a liquid sample. In the illustrated apparatus there is a plurality of indicator zones **106*a*** and **106*b***. At least one of such indicator zones contains the complementary member of a specific binding pair

where the other member is the analyte of interest or derivative thereof, i.e., a substance which binds specifically to the analyte, e.g., an antibody-antigen. In the apparatus of FIGS. 1–3, there is also provided an absorption zone 104 which is adapted to absorb liquid flowing through the membrane from the sampling zone.

The absorption zone 104 may be of sufficient capacity to permit the required flow from the sample zone through the indicator zone(s) to be retained in the membrane. Alternatively, an absorbent body 108 which may be a pad of cellulose fibers, a cellulose sponge, or any other high-capacity hydrophilic material capable of absorbing liquid may be provided.

In the particular apparatus shown, pad or disc 110 may be used as a source of supplementary reagent. The disc may also be a non-bibulous membrane or may be a liquid absorbent, such as filter paper. For example, in an ELISA assay, disc 110 may contain enzyme-labeled antibody, which dissolves in the sample and couples with an analyte antigen. The complex is then retained in the indicator zone by additional antibody capable of binding the analyte-enzyme labelled complex which is formed.

The invention method is conducted using the apparatus of FIGS. 1-3 by introducing a liquid sample in the application zone 102. The liquid flows laterally without retention through the indicator zone 106*a* and 106*b* and into the absorption zone 104 and/or an adsorptive pad 108. Unbound species are washed from the indicator zone, if necessary, or desirable, to make reading easier. A developer or other reagent is added, if required, to develop a signal which can be detected visually, instrumentally or otherwise. The signal is relatable to the amount of the analyte. Additional steps and variations in the order of carrying out of the steps will depend upon the particular binding pair under consideration, the particular label or marker, etc., as required or desired depending on the specific mode of developing the detectable signal. In some instances, only two steps are required. For example, in one embodiment, whole blood is simply introduced to the sample application zone, unbound or unentrapped cells are washed through the indicator zone, and red blood cell color is sufficient to permit direct visual reading of the assay.

multiple analytes

Multiple analytes in a single sample can be determined with a single apparatus by providing multiple indicator zones so that each indicator binds only one analyte.

Referring again to FIGS. 1-3, a marketable kit is shown. The kit includes, in addition to the lateral flow non-bibulous membrane 100, a base 120 which has a surrounding lip 122 forming a bottom reservoir when the kit is in use. A flange 124 extends upwardly from the lip and permits easy attachment to the top which will be described. Notches 126 at one end and 128 at the other end provide for alignment of the top and attachment of the top to the base. Inside the bottom reservoir 130 the base structure forms a support 131 for the application zone 102 of the membrane 100 and walls 134 and 136 which include a solid support or plurality of supporting columns 134*a* and 136*a* respectively on the walls which serve to support the edges of the membrane 100 in the indicator zone(s) 106 thereof. The reservoir and walls are so constructed and configured as to receive inside the reservoir the absorbent body 108 which, in the illustrated example, is a rather large U-shaped body to provide ample excess capacity in the event the operator uses excess wash, developer or other reagent solutions.

The disc 110 in addition to or instead of carrying a reagent, can act as a filter for the application zone 102 of the membrane 100 removing large particles from the sample. The disc is, however, optional. The liquid sample is introduced to the pad 110 or directly to the zone 102 by a pipette, dropper or other device.

The wash or buffer solutions and the reagents may be introduced through the pad 110 or directly to and through the application zone 102 to and through the indicator zone(s) 106. The analyte of interest, if present, is bound specifically to the membrane in one or more of the indicator zones. The wash or buffer solutions and, if used, the other reagent solutions, may also be introduced directly into the indicator area.

The top or cover 140 is constructed and adapted to fit snugly with the base 120 and includes a flange 142 which fits in close engagement with the flange 124 on the base 120. The top forms and defines an opening therethrough in the form of a well 132 which may, as shown, have chamfered or beveled sides, or may be in any shape or size or configuration of convenience. The well 132 provides access to the disc 110 for introducing sample and wash and reagents. One or more "indicator" apertures 146 e.g., 146*a* and 146*b* are also formed and defined by the top 140 permitting viewing of the "indicator" zone(s) on the membrane. These apertures may be formed of or covered with thin, clear or translucent coverings, if desired, it being necessary only that one be able to see or otherwise "read", i.e., detect, the color, radioactivity or fluorescence, or other signal, in the indicator zone. The apertures, or other indicator covering structures, may be in special shapes such as a "plus" sign, a "minus" sign, a circle, or in any other configuration to provide access to the indicator zone. The shape of the indicator access is simply a convenience and not of operational significance.

FIGS. 4–8 show a self-contained kit, i.e., a kit which does not require additional reagents or equipment. The critical membrane 200 is shown as an elongate strip, which has an application zone 202, a flow-through or absorbent zone 204 which accepts liquid laterally traversing the membrane through the "indicator" zone or a plurality of indicator zones 206, e.g., 206*a* and 206*b*. At least one of such indicator zones has bound therein a member of a specific binding complex comprising the analyte of interest and substrate which binds to analyte. An absorbent body 208 which is of a high-capacity hydrophilic material capable of absorbing the liquid is also shown. Disc 210 functions as described respecting pad 110.

The kit includes, in addition to the non-bibulous capillary flow membrane, pad and absorber described, a base 220 which has a surrounding lip 222 forming a bottom reservoir when the kit is in use. A flange 224 extends upwardly from the lip and permits easy attachment to the top which will be described. Notches 226 at one end and 228 at the other end provide for alignment of the top and attachment of the top to the base. Inside the bottom reservoir 230 the base structure forms a support 232 for the sample application zone 202 of the non-bibulous lateral flow membrane 200 and walls 234, 236, and 238 which include a plurality of supporting columns 236*a* and 238*a* respectively on the walls which serve to support the edges of the membrane 200 in the indicator zone(s) 206 thereof. The reservoir and walls are so constructed and configured as to receive inside the reservoir the absorbent body 208 which, in the illustrated example, is an absorbent body of sufficient size

and shape and composition to absorb all, or substantially all of the liquids which would be used in a normal assay. The absorbent body may be of any moisture absorbent material, cellulosic fibers, fibers or particles of cellulose esters, etc. The walls 236 and 238 and the bottom 220 generally define a space for receiving two vials 240 and 242 which are of glass, polymer or other crushable or openable material. An inexpensive polymeric "vial" may be formed of any suitably inert polymeric membrane, e.g., polyethylene, polyvinyl chloride, polycarbonate, etc., of the desired size and shape having a weakened or thin area in a portion adjacent the sample zone which will rupture upon the application of pressure. A simple glass vial may, of course, be used. In this example, and this is but one illustrative example, the vial 240 contains a wash solution and the vial 242 contains a buffer solution.

The top or cover 260 is constructed and adapted to fit snugly with the bottom 220 and includes a flange 262 which fits in close engagement with the flange 224 on the bottom 220. The top forms and defines an opening therethrough in the form of a well 264 which may, as shown, have chamfered or beveled sides, or may be in any shape or size or configuration of convenience and, in this embodiment, includes surrounding skirt 266 which directs the liquid to the sample receiving pad. The well provides access to the pad 210 for introducing sample and wash and reagents to the non-bibulous capillary flow membrane 200 for carrying out the assay. One or more "indicator" apertures 268 are also formed and defined by the top 260 permitting viewing of the "indicator" zone(s) on the non-bibulous capillary flow membrane. These apertures 268 may be formed of or covered with, thin, specially formed, clear or translucent coverings, or be openings, it being necessary only that one be able to see or otherwise detect the color.

In this example, a pair of crusher buttons 270 and 272 joined by a flexible strip 274 are positioned on the sides of the top and are moveable downwardly by application of force by the user, e.g., by pressing the button. Each of the buttons is so constructed, configured and positioned that such downward movement, as viewed in the figure, will cause a portion of the button to engage one of the vials and crush, rupture or otherwise open the vial. While this is a convenient mechanism, it is not necessary to the invention that the precise mechanism be as described in the example. For example, the buttons may be independent of each other, there may be three or more vials and three or more buttons, etc. or other breaking and/or opening mechanisms may be used. The kit of FIGS. 4–8 is a preferred but only illustrative embodiment.

A simple but very effective form of the invention apparatus is shown in FIG. 9. This apparatus comprises a sheet of the isotropic lateral flow, non-bibulous, membrane having, near the center thereof, an application zone S which is adapted, constructed and treated as necessary to receive and absorb for example, a cell-bearing liquid sample, e.g., blood. At least one indicator zone A and, in this embodiment, a plurality of indicator zones A, B, C and D are provided equidistantly spaced (in this illustration) from the sample application zone S. In this illustration there is additional membrane beyond each indicator zone as an "absorbent zone" to encourage flow of sample. The geometry is, of course, arbitrary; the requirements being that there be an application zone capable of receiving from an external source an aqueous liquid, optionally but preferably an absorption zone capable of permitting additional flow of liquid in the membrane, and intermediate to, and spaced from, each of the application and absorption zones, at least one indicator zone; the indicator zone being different from the remainder of the membrane in that to it is affixed one member of a binding pair, e.g., an antibody or a receptor, or an antigen.

As shown in FIG. 10, a substantially liquid impervious film or layer 314 may be bonded to one side of the membrane 312, there being formed a sample receiving aperture S and one or more apertures at A and C, for example for viewing indicator zones. Additionally, another liquid impervious film or layer 316 may be bonded to the other side of the membrane to form a sandwich. The liquid impervious layers are, e.g., polyethylene or other polymer film, or paper which has been coated or treated, e.g., with wax or a hydrophobic polymer such as silicone.

In one manner of conducting the method with this illustrated apparatus, sample is applied to S and allowed to flow through A–D to which is affixed a member of a binding pair. Sufficient time of incubation (30 sec–several minutes) is used in order to permit the analyte to react with a binding partner affixed in one or more indicator zone A–D. This is followed by washing conveniently by applying wash solution to S, and then by detection of bound analyte in A–D.

Illustrative Applications of the Invention Apparatus

Analysis of Blood Samples

One major application of the invention apparatus is in blood typing and characterization. In the assessment of whole blood, red blood cells can be used as a detection reagent, whether directly bound by their surface antigens to the binding pair member in the indicator zone or entrapped by a complex formed between members of a different binding pair in said zone. Both soluble and particle-borne analytes are capable of forming such complexes.

Blood Typing and Characterization

Thus, for example, the invention apparatus and methods are useful for determining the blood grouping of erythrocytes (A, B, 0, Rh) or cell typing with leucocytes or platelets (HLA or Pl^{A1}).

By way of background, cells found in mammalian blood are, principally, erythrocytes (red blood cells or red blood cells), thrombocytes (platelets), granulocytes, monocytes, T lymphocytes and B lymphocytes (leucocytes). Specific binding pair immunochemistry of erythrocytes is important in whole blood transfusions in which the ABO and Rh blood group matching is required. Matching of antigens associated with other blood cell types is also important in transplantation and in individual identification—e.g., HLA typing.

The *TECHNICAL MANUAL of the American Association of Blood Banks*, American Association of Blood Banks, Arlington, Virginia, describes the most widely used nomenclature. The most common blood groups are the ABO and the Rh blood group systems. A and B refer to antigens present on red blood cells, type O does not contain these antigens. Rh positive blood cells contain the "Rh" or "D" antigen. The reality is somewhat more complex, however as a first approximation, the following immunological binding relationships are defined.

ABO Blood Grouping*

ABO Blood Grouping*					
Cell Reaction with Antibodies to Blood Group Antigens		Reaction of Serum Tested Against Blood Group Cells			ABO
Anti-A	Anti-B	A Cells	B Cells	O Cells	Group
−	−	+	+	−	O
+	−	−	+	−	A
−	+	+	−	−	B
+	+	−	−	−	AB

*Taken from the Technical Manual of the American Association of Blood Banks.

Rh Blood Grouping

Rh Blood Grouping	
Reaction with Rh Blood Group Antigen D	Rh Group
+	Positive
−	Negative

Reference is made to the aforesaid Technical Manual and to more comprehensive works for a more complete description of blood groupings and related nomenclature. Specific binding ABO dipsticks are disclosed by Plapp, F. V., et al, *The Lancelet*, June 28, 1986, pp. 1465–1466.

Platelet antigens, Pl^{A1} and Pl^{A2}, Ko^{a} and Ko^{b}, and Pl^{E1} and Pl^{E2}, and other less common platelet antigens naturally occur on the surface of platelets in humans at a greater or lesser frequency. For example, Pl^{A1}, which is associated with the control of bleeding episodes, occurs in about 97% of the population and Pl^{A2} occurs in about 27% of the population.

Human Lymphocyte Antigens (HLA) and other Pl^{A} antigens occur on the surface of platelets. Pl^{A} antigens are specific to platelets, however, while HLA are found on all nucleated cells in the body, those of solid tissue and most of the circulating blood cells, except red blood cells. The major human histocompatibility complex (MHC) is a cluster of genes denominated HLA-A, HLA-B, HLA-C, HLA-D and HLA-DR which produce antigens. HLA-A, HLA-B and HLA-C antigens constitute the major transplantation antigens. HLA-D and HLA-DR are believed to be involved in immune responsiveness.

HLA typing is fundamental to paternity determinations, therapy involving blood components, organ transplant and transfusion compatibility determinations, and in other medical and scientific studies.

In blood therapy, a high percentage of patients receiving repeated transfusion of random-donor platelet transfusions become refractory to further random-donor platelet transfusions, but single donor HLA-matched platelets can be of benefit in treating many of these refractory patients. HLA-A, HLA-B, and HLA-C antigens are considerably more important in selecting single-donor platelets than ABO antigens which, at most, are weakly expressed on platelets and HLA-D and -DR which are not present on the platelet surface. Perfect single-donor HLA-platelet matching is, at best, an arduous process, requiring a very large donor pool and a great many matching tests. It would, therefore, be an advance of great importance that the matching tests be carried out quickly and inexpensively.

The standard technique used to detect HLA-A, -B, -C, -D, -DR and -DQ antigens is the microlymphocytotoxicity test. The HLA-D system is determined by cellular events in mixed lymphocyte culture (MLC) tests. Primed lymphocyte typing (PLT) and Cell-Mediated Lympholysis (CML) tests are also used in HLA antigen testing. These methods and other current methods for HLA typing require sophisticated and expensive equipment and highly trained technicians; see "HLA Techniques for Blood Bankers and Technical Manual of the American Association of Blood Banks", (1984), American Association of Blood Banks, Arlington, VA, USA.

Other cytotoxicity based HLA typing methods have also been reported; see, e.g., Grumet, et al, U.S. Pat. No. 4,471,056; Terasaki, et al, U.S. Pat. No. 4,599,315; and U.S. Pat. No. 4,324,026.

Specific binding reaction HLA assays have also been described, DeFreitas, U.S. Pat. No. 4,492,760 (assay for HLA-D typing using monocytes which had been contacted with a particular antigen and then incubated with antigen-specific T lymphocytes or antigen-specific T cell hybridomas) Engleman, et al, U.S. Pat. 4,634,666 (immuno-fluorescent assay for HLA antigens using monoclonal antibodies), Old, et al, U.S. Pat. No. 4,650,756 (monoclonal antibodies which bind to HLA); Eisinger, R. W. and R. A. Eatz, Program and Abstract First Annual ASM Conference on Biotechnology, 1986.

Platelet antibody assays generally have been described by Schiffer, C. A., in "A Seminar in Antigens in Blood Cells and Body Fluids", Bell, C. A., ed., Washington, D.C. American Association of Blood Banks; 1980:189–208) and Brand, A., et al, (*Blood* (1978) 781–788) and many others.

A summary of some of the available non-red blood cell surface markers is summarized in the table below:

TABLE IV

	Differentiation of Lymphocytes		
Marker	T Cell	B Cell	Macrophage
Specific Surface Antigens	OKT, Leu	HBLA	OKM1
Antigen Binding Receptor	V Region	Ig	—
Receptors for:			
sRBC (E-rosette)	+	−	−
IgG Fc (EA-rosette)	+	+	+
IgM Fc (EA-rosette)	+	−	−
C3b (EAC rosette)	−	+	+
Measles Virus	+	−	−
Epstein Barr Virus	−	+	+
HLA-A,B,C Antigens	+	+	+
HLA-D/Dr Antigens	+/−	+	+

Reference: Herscowitz, "Immunophysiology: Cell Function and Cellular Interactions in Antibody Formation" in Immunology III edited by J. A. Bellanti, W. B. Saunders Co., Philadelphia, 1985.

As further described below, the invention method is particularly useful in assessing the presence or absence of these cell surface markers in blood. Antibodies to the cell surface marker of interest are placed in an indicator zone and a sample of blood to be tested is placed in the application zone in sufficient volume to flow past the indicator zone(s). Multiple indicator zones, each having affixed antibody for a different cell surface marker, may be used. A radial arrangement, such as that of FIG. 9 may be convenient. All of the indicator zones will appear red. After a suitable incubation period, usually less than a minute, wash solution is then flowed past the indicator zone(s), most conveniently by supplying the wash solution to the application zone in sufficient amount to wash away all unbound or unentrapped red

blood cells. A control "blank" zone equidistant with the other indicator zones from the application zone is useful in verifying the correct quantity of wash; this zone will appear clear when sufficient wash is added. The results can then be read: indicator zones which have affixed antibodies to cell surface markers present in the sample will appear red; those with antibodies to markers not present will be clear. If the cell surface markers are on the red blood cells, the red blood cells will be directly bound to the fixed antibody in the zone; if the markers are on platelets or leucocytes, the red blood cells will be entrapped in the complex formed by these cells with the fixed antibody.

Determination of Soluble Blood Components

It has been found that complexes formed between fixed antibody (or other binding partner) and soluble blood components are also capable of entrapping red blood cells to generate a red color in the reaction zone. Thus, soluble materials in the blood are assessable in an analogous way. For example, proteins and nucleic acid associated with infection are capable of antibody recognition or recognition by other binding partners. Other blood components such as antibodies or lipoproteins can also be detected.

For example, during the initial stages of Hepatitis B Virus infection, the Hepatitis B surface Antigen (HBsAg) is detected in the serum, followed by a measurable amount of Hepatitis B envelope Antigen (HBeAg) and antibody to Hepatitis B core Antigen (anti-HBcAg) titer during the acute stage of the disease. Production of anti-HBeAg and anti-HBsAg occur later. All of these are detectable by binding assay protocols, as are indications of Herpes Simplex I and II, HIV and cytomegalovirus infections.

Other important soluble blood components include lipoproteins which are conjugated proteins in which the prosthetic groups are lipids. These are also found in cell cytoplasm. Assay of lipoproteins is of interest, because there is a direct correlation between the blood level of lipoproteins, especially low density lipoproteins, and the risk of cardiovascular disease, such as atherosclerosis. Apolipoprotein A-1 and Apolipoprotein B in serum are key indicators and are considered to play a key role in the progress and management of cardiovascular diseases.

For detection of soluble components in whole blood, in the typical protocol, as for cell surface markers, whole blood is used as the sample, and a material specifically reactive with the soluble analyte is fixed in an indicator zone. Multiple indicator zones can be used, each with a specifically reactive complementary member for binding to the soluble analyte.

For example, to test for lipoprotein, a whole blood sample of about 4–5 drops is applied to the application zone of the apparatus of FIG. 9 wherein the indicator zones A, B, C or D contain antibody to the lipoprotein(s) or lipoprotein derived or related component(s) to be determined. Sufficient volume is applied to allow for lateral migration through the membrane through the indicator zones. A brief incubation of from a few (e.g., 15) seconds to several (e.g., 30) minutes, typically between one and two minutes at room temperature, permits the binding of the lipoprotein to the member of the specific binding pair affixed to the indicator zone. For example, one might affix anti-apolipoprotein A-1 in a first zone, anti-apolipoprotein B in the second, anti-apolipoprotein E in a third, etc. After sample addition, a lattice network is formed between the affixed antibodies and the lipoprotein which entraps the erythrocytes from the blood. Thus, when wash is added to the application zone and flows laterally through the indicator zones, those containing bound analyte retain the red blood cells and appear red. In those which do not, the red blood cells will be washed away and these indicator zones will appear clear.

Use of Added Detectable Particles

It will be evident that while assay of whole blood furnishes a convenient source of visible particles, non-blood samples could also be used with the addition of visible particles. For example, red blood cells or colored latex beads or the like could be used to supplement a serum sample, spinal fluid, or urine sample. The particles can be added along with sample, or subsequent thereto.

For instance, for detection and titration of antibody to viral components in serum, viral antigen components or the denatured virus itself can be affixed to an indicator zone. While whole blood could be used, as described above, in the alternative, the serum can be tested directly when supplemented with red blood cells or other colored particles. Conversely, to detect a viral or bacterial antigen, a monoclonal or polyclonal antibody directed to the antigen is fixed to the indicator zone. As before, the sample modified to contain detectable particles is applied at the application zone and flows laterally through the indicator zone. In an alternative, the particles can be added subsequent to sample. In another alternative, the particles could be impregnated into the application zone during manufacture. For example, latex beads could be lyophilized in place in the application zone. The complex resulting in the indicator zone provides a lattice network which entraps the particles, giving a positive reaction, e.g., a visible color at the indicator zone rather than a clear zone.

In general, then, serum, urine, spinal fluid, plasma, or other body fluid, or liquid from other sources, such as manufacturing lots in the pharmaceutical, food or cosmetic industry, effluents from industrial processes, or any liquid suspected of containing a specific analyte can be used as sample. The analyte is detected in the invention method by providing a fixed binding pair member, capable of binding analyte or derivative thereof, in an indicator zone, and by providing a suspension of detectable particles along with the sample, or subsequent to the passage of sample through the indicator zone. The detection is by appearance of the detectable particles, after washing, in the indicator zone.

Applications in general include monitoring antigens and antibodies during stages of infection, and for general diagnosis and monitoring of therapy. Hormones, enzymes, other blood proteins, or tumor antigens shed into the bloodstream or other body fluids following chemotherapy or radiation therapy may also be monitored.

Detection Using Non-Particulate Label

While detectable particulate entrapment, especially visible particle entrapment, is a convenient method, other specific binding assays are adaptable to the method and apparatus of the invention, as set forth above. For example, the standard hCG ELISA test for pregnancy can be adapted to this method either with separate or self-contained reagents.

Generally, for the hCG assay, a urine sample is applied to the application zone and allowed to flow through an indicator zone containing anti-hCG antibodies. After washing, a second, labeled anti-hCG is then applied to the indicator zone either directly or by flow from the application zone, and, if needed, followed by developing reagents. If the label is an enzyme, the developing reagents will include the enzyme substrate. Alternatively, the urine sample can be treated first with labeled murine anti-hCG and the mixture flowed laterally through an indicator zone to which is fixed a monoclonal anti-hCG Ig preparation which binds to a different epitope. A variety of protocols can be used.

The reagents can be added independently as in the apparatus of FIGS. 1-3, or they can be self contained, as in that of 4-8. In the latter case, for example, the indicator zone may contain fixed anti-hCG; a vial included in the apparatus may contain enzyme-labeled anti-hCG.

"Reverse" Assay Protocols

In addition to the standard method of supplying sample to the application zone, the protocol can, of course, be reversed by affixing the analyte from a sample into an indicator zone and supplying detecting reagent by lateral flow from the application zone. For example, the presence of a specific antibody or antigen in serum can be detected by affixing the serum sample to the indicator zone, followed by addition to the application zone of a labeled form of the complementary antigen or antibody, respectively. In this manner, multiple samples can be assayed in the apparatus of FIG. 9-10, or of other design, by utilization of the multiplicity of indicator zones.

By way of illustration, four different serum samples to be tested for the presence of herpes virus protein are affixed individually to zones A-D by drying the samples applied to these indicator zones. The application zone is then employed to effect the flow of a reagent solution containing labeled anti-hepatitis antibodies through the membrane and past the indicator zones. Incubation time is allowed to permit any viral protein in the samples to bind to antibody, and a wash solution is then applied to the application zone to wash away antibody not bound to the indicator zones. If the label is an enzyme, this is followed by a detection reagent such as a substrate mixture. Alternatively, non-labeled antibodies can be used as the initial reagent, followed by addition of, for example, colored particles as described above, or a labeled antibody reactive either with the viral antigen or with the antibody reagent. The presence of label showing viral antigen in a particular zone indicates the presence of viral antigen in the relevant sample.

In an alternative protocol, for example, urine samples to be tested for the presence of hCG are placed individually in indicator zones A-D and dried. A reagent containing labelled anti-hCG antibody is supplied to the application zone and permitted to flow through the membrane past the various indicator zones A-D. After a suitable incubation period, a wash solution is applied at the application zone S, or in the alternative, directly to the indicator zones. The presence of label in an indicator zone, then, demonstrates the presence of hCG in the sample.

In another example, compatibility for platelet transfusion between potential donors and a recipient can be determined. Serum from the donor can be placed on multiple test units in an indicator zone and dried. A whole blood test sample from a potential donor is supplied to the application zone and permitted to laterally flow through the indicator zone. After a suitable incubation, a wash solution is applied in the application zone. A lack of signal is indicative of compatibility between the recipient and the tested donor. A positive reaction (red spot in the indicator zone) is indicative of an incompatible match due to antibodies present in the recipient's serum recognizing and binding the antigens present on the potential donor's platelets. The incompatible match results in exclusion of this donor for the recipient. Multiple donors can be screened using multiple test units.

In an alternative form, serum samples from several recipients requiring platelet transfusions could be tested against a single potential donor to determine compatibility/incompatibility. Serum from each recipient would be spotted into individual indicator zones and dried. A whole blood test sample from a potential donor could be applied at the application zone and compatibility with any of the recipients determined. This type of antibody screen assay would be applicable for platelet, red blood cell and other cell components transfusion compatibility or crossmatch testing.

Semi-Quantitative Assay

By varying the amount of specific binding pair member in a multiplicity of indicator zones on the membrane, the assay can be made semiquantitative. For example, serial dilutions of antibody can be used in zones A-D of the apparatus of FIG. 9-10, and sample to be analyzed for antigen provided to the application zone. Labeling can be by subsequent addition of, e.g., labeled Ig capable of binding to the complex, or by the use of detectable particles.

In this assay, higher concentrations of analyte are able to show detectable reactions to lower amounts (higher dilutions) of affixed binding pair member. Thus, by calibrating serial dilutions of a binding pair member preparation, affixed over a series of indicator zones, to varying concentrations of analyte, an at least semi-quantitative result can be obtained.

EXAMPLES

The following examples illustrate, but do not limit the invention.

EXAMPLE 1

Determination of Platelet Antigen Components

The illustrative apparatus of FIGS. 9 and 10 was used to determine the platelet surface antigen in blood samples. There are two major groups of glycoproteins present on the platelet cell surface; platelet-specific antigens (Pl^{A1}, Pl^{A2}, Pl^{E}, Lek, Bak, Duzo) and platelet-associated antigens (HLA-A, -B, -C antigens), which are also found on other cell types.

Referring to FIG. 9, whole blood is applied to the application zone, S, the sample components, including platelets and red blood cells, laterally flow through the membrane passing the indicator zones, each of which contains antibody specific to a platelet antigen. In zones containing antibody specific for a platelet marker present in the sample, the bound platelets form a lattice network which entraps the red blood cells in the test sample. This is evidenced as a red color in the zone following the addition of wash buffer to remove un-

bound cells. A clear or white zone is indicative of a lack of the relevant marker.

Pl^{A1} Antigen

Extra Fine grade and Fine grade Ultra High Molecular Polyethylene porous plastic (Porex Technologies Inc., Fairburn, GA) was cut into two-inch long×0.5 inch strips. Five microliters of antibody with demonstrated specificity to the Pl^{A1} antigen (Lot KRO, Blood Center of Southeastern Wisconsin) was spotted approximately one inch from the end of the porous plastic strip. The positive control for this assay consisted of spotting 5 μl of Rabbit Anti-Thrombocyte Membrane (Dako, Lot 035) onto duplicate strips. This antibody binds all platelets regardless of Pl^{A1} phenotype. The strips were dried at either 37° C. or 25° C. for 15 minutes. The dried strips were stored at 25° C. with desiccant capsules (Dricap, Multiform Desiccants, Inc.).

Prior to performance of the assay, absorbent pads (Schleicher and Schuell, #A300) were attached to one end of the porous plastic strip. Platelets were previously typed for Pl^{A1} antigen by the immunofluorescence procedure (St. Louis. American Red Cross). Blood samples were collected in ethylenediamine tetraacetic acid (EDTA) anticoagulant. Four to five drops (100–125 μl) of whole blood were added to the sample application end of the porous plastic strip. This ensured a sufficient volume of blood to flow laterally past the antibody spotted region. Following a three minute incubation at 25° C., 16–20 drops of wash reagent were added to the sample application end. This caused the unbound cells to flow from the antibody spotted region to the absorbent pad area.

A positive reaction was characterized by a red dot at the site of antibody application. This results when the porous plastic affixed antibody (Anti-Pl^{A1}) antibodies recognize the Pl^{A1} antigen present on the platelet cell surface and bind the platelets. This subsequently forms a lattice which entraps the red blood cells in the test sample. A negative reaction is identified as a clear or white region at the antibody application site.

Blood samples from three donors identified as being Pl^{A1} positive by immunofluorescence produced a positive reaction with an Anti-Pl^{A1} serum (Lot KRO); negative reactions were obtained from two Pl^{A1} negative donors. (A positive control in the assay used Rabbit Anti-Thrombocyte (Dako Lot 015) as a binding antibody in the reading zone; it binds thrombocytes regardless of Pl^{A1} phenotyping. Blood samples from all five donors tested produced positive reactions.)

Results

	Results:		
Donor ID	Anti-Pl^{A1} status*	Positive Control	RBC Lateral Flow
ABO#211	Negative	Positive	Negative
ABO#132	Positive	Positive	Positive
ABO#131	Positive	Positive	Positive
ABO#130	Negative	Positive	Negative
ABO#129	Positive	Positive	Positive

*As determined by immunofluorescence testing, St. Louis Chapter American Red Cross.

EXAMPLE 2

ABO Rh Grouping

Fine grade Ultra High Molecular Weight Polyethylene porous plastic (Porex Technologies, Inc., Fairburn, GA) was cut into 1.5 inch long×1.5 inch blocks. The block was assembled between two polystyrene pieces slightly larger than the porous plastic block. Five wells or windows were punched out of the top polystyrene piece at a distance of approximately 0.25 inches from the center of the porous plastic block. Four microliters of either monoclonal Anti-Blood Group A (Ortho Diagnostics, Lot 109D), monoclonal Anti-Blood Group B (Ortho Diagnostics, Lot BBB 506A) or Anti-Blood Group Rh (Ortho Diagnostics, Lot DN 298A) were spotted into individual wells. The positive control for this assay consisted of spotting 4 μl of Rabbit Anti-Erythrocyte Membrane (Dako, Lot 015) into a fourth well. This antibody binds all erythrocytes regardless of ABO Rh blood type. A fifth well serves as the negative control well and is not spotted with any antibody, but serves to demonstrate the lack of nonspecific reactions as well as the complete removal of unbound red blood cells from the test area. The spotted units were dried at 37° C. for 30 minutes. The dried units were stored at 25° C. with desiccant capsules (Dricap, Multiform Desiccants, Inc.).

Blood samples were collected in EDTA acid citrate dextrose, or ACD; citrate phosphate dextrose, or CPD; citrate phosphate dextrose adenine, or CPDA-1; or Adsol, a brand name of Baxter-Travenol, Chicago, Illinois; or heparin anticoagulants. Four drops (100–125 ul) of whole blood were added to the center sample application well. This ensured a sufficient volume of blood to flow laterally past the antibody spotted regions. Following a thirty second incubation at 25° C., 16–20 drops (approximately 0.5–0.6 ml) of wash reagent were added to the center sample application well. This caused the unbound cells to flow from the antibody-spotted (indicator) region to the remaining (absorbent) region of the porous plastic block.

A positive reaction was characterized by a red dot at the site of antibody application. This results when the antibody affixed to the porous plastic recognizes the appropriate erythrocyte cell surface antigen and binds the red blood cells. A negative reaction is identified as a clear or white region at the antibody application site.

The following results were obtained:

Blood Group*	Total Samples Tested	Number Correctly Identified by RBC Lateral Flow
A	65	65
A_X	7	7
B	47	47
O	88	88
AB	31	31
Rh Positive	141	141
Rh Negative	98	98
D^u Positive	4	4

*Determined by Routine Tube Testing hemagglutination procedures

EXAMPLE 3

ABO Reverse Grouping Immunoassay

The serum of type O blood contains anti-A and anti-B immunoglobulins, that of type B blood contains anti-A; that of type A blood, anti-B. Thus blood can also be typed by detecting these antibodies in serum. The test sera are spotted into indicator zones and fixed and assayed by applying whole blood of known type to the application zone.

Extra Fine grade Ultra High Molecular Weight Polyethylene porous plastic (Porex Technologies, Fairburn, Ga) was cut into two-inch long×0.5 inch strips. Five ul of serum from a Blood Group A donor (MH), a Blood Group B donor (RWE), a Blood Group O donor (ABO #31) and a Blood Group AB donor (Serologicals #44) were spotted approximately one inch from the center on duplicate porous plastic strips. The positive control for this assay consisted of spotting 5 ul of Rabbit Anti-Erythrocyte Membrane (Dako, Lot 015) at a second site on each strip. The positive control antibody binds all red blood cells regardless of blood grouping.

The strips were dried at either 37° C. or 25° C. for 15 minutes The dried strips were stored at 25° C. with desiccant capsules (Dricap Co.).

Prior to performance of the assay, absorbent pads (Schleicher and Schuell, #A300) were attached to both ends of the porous plastic strip. Red blood cells which had been previously typed using routine tube testing hemagglutination procedures were collected in ethylene-diamine tetraacetic acid (EDTA) anticoagulant. Four to five drops (100–125 ul) of whole blood from identified Blood Group A or Blood Group B donors were added to the center of each porous plastic strip. This ensured a sufficient volume of blood to laterally flow past the antibody spotted regions. Following a one minute incubation at 25° C., 6–8 drops of wash reagent were added to the center of the strip. This caused the unbound cells to flow from the antibody spotted region to the absorbent pad area.

A positive reaction was characterized by a red dot at the site of antibody application, e.g., red color will appear for blood type O when tested with A and B red blood cells; for blood type B with group A red blood cells; and for blood type A with group B red blood cells. Type AB serum will produce clear (negative) reactions with both group A and group B red blood cells.

The results are as follows:

Donor ID	RTT Grouping*	Positive Control	RBC Lateral Flow A Cells	RBC Lateral Flow B Cells
MH	Group A	Positive	Negative	Positive
RWE	Group B	Positive	Positive	Negative
ABO#31	Group O	Positive	Positive	Positive
Sero.#44	Group AB	Positive	Negative	Negative

*As determined by standard Routine Tube Testing Procedures.

EXAMPLE 4

A Red Blood Cell Typing (Unexpected Red Blood Cell Antibody Screen) Immunoassay

Extra Fine grade Ultra High Molecular Weight Polyethylene porous plastic (Porex Technologies Inc., Fairburn, GA) was cut into two-inch long×0.5 inch strips. Five microliters of ANTI-C (Ortho Diagnostics, Lot CS 157A) were spotted approximately one inch from the center of the porous plastic strip. The positive control for this assay consisted of spotting 5 ul of Rabbit Anti-Erythrocyte Membrane (Dako, Lot 015) at a second site on the strip. The positive control antibody binds all red blood cells regardless of blood grouping. The strips were dried at either 37° C. or 25° C. for 15 minutes. The dried strips were stored at 25° C. with desiccant capsules (Dricap Co.).

Prior to performance of the assay, absorbent pads (Schleicher and Schuell, #A300) were attached to both ends of the porous plastic strip. Red blood cells were previously typed using Routine Tube Testing hemagglutination procedures (University of California, San Diego Medical Center). Blood samples were obtained from collection unit segments containing CPD anticoagulant. Four to five drops (100–125 ul) of whole blood from a donor were added to the center of the porous plastic strip. This ensured a sufficient volume of blood to laterally flow past the antibody spotted regions. Following a 1.5 minute incubation at 25° C., 16–20 drops of wash reagent were added to the center of the strip. This caused the unbound cells to flow from the antibody spotted region to the absorbent pad area.

A positive reaction was characterized by a red dot at the site of antibody application. This results when the antibody affixed to the porous plastic recognizes the specific red blood cell antigen present on the cell surface and binds the red blood cell. A negative reaction is identified as a clear or white region at the antibody application site.

Results

Donor ID	RTT Grouping*	Positive Control	RBC Lateral Flow
KC	C positive	Positive	Positive
PB	C negative	Positive	Negative

*As determined by standard Routine Tube Testing procedures, UCSD Medical Center.

EXAMPLE 5

Tissue Typing Immunoassay

A. Extra Fine grade and Fine grade Ultra High Molecular Weight Polyethylene porous plastic (Porex Technologies Inc., Fairburn, GA) was cut into two-inch long×0.5 inch strips. Five microliters of Rabbit ANTI-Thrombocyte Membrane (Dako, Lot 035) were spotted approximately one inch from the center of the porous plastic strip. The anti-thrombocyte is specific to binding platelets and thrombocytes. The positive control for this assay consisted of spotting 5 ul of Rabbit Anti-Erythrocyte Membrane (Dako, Lot 015) at a second site on the strip. The positive control antibody binds erythrocytes, but does not bind to platelets. A third region of each strip was designated as the Negative control. This area was not spotted with an antibody, rather it served to demonstrate appropriate removal of unbound red blood cells from the strip and lack of non-specific reactions. The strips were dried at 37° C. for 15 The dried strips were stored at 25° C. with desiccant capsules (Dricap Co.).

Prior to performance of the assay, absorbent pads (Schleicher and Schuell, #A300) were attached to both ends of the porous plastic strip. Blood samples from two group O donors were obtained in EDTA anticoagulant. Four to five drops (100–125 ul) of whole blood from a donor were added to the center of the porous plastic strip. This ensured a sufficient volume of blood to laterally flow past the antibody spotted regions. Following a 30 second incubation at 25° C., 4–6 drops of wash reagent were added to the center of the strip. This caused the unbound cells to flow from the antibody spotted region to the absorbent pad area.

Assay specificity was tested by preparation of Platelet Rich Plasma from aliquots of each test sample to remove the thrombocytes and platelets. The remaining packed red blood cells were subsequently washed three

times in PBS. Four drops of each cell suspension were subsequently tested as described above.

The addition of 100 ul of the Platelet Rich Plasma to the washed red blood cell fractions was performed to demonstrate the cell specificity of the reactions. A positive reaction was characterized by a red dot at the site of antibody application. This results when the antibody affixed to the porous plastic recognizes antigens present on platelet and thrombocyte cell surfaces and binds these cells thus forming a lattice network entrapping red blood cells in the test sample. A negative reaction is identified as a clear or white region at the antibody application site.

Whole blood samples from two donors, previously typed by lymphocytotoxicity as HLA-A2, produced positive reactions; one donor who was typed HLA-A2 negative gave a negative reaction. All three donors, regardless of HLA profile, gave positive reactions with the Anti-Thrombocyte positive control. The above assay provides a simple alternative to current methods to provide platelet type evaluation to crossmatch donor/receptor in transfusions.

Results

Donor ID	Anti-Thrombocyte	Anti-Erythroctye	Negative Control
ABO#45(Gr.O)	Positive	Positive	Negative
ABO#45(Gr.O)-less PRP	Negative	Positive	Negative
ABO#45(Gr.O)-Add Back	Positive weak	Positive	Negative
ABO#208(Gr.O)	Positive	Positive	Negative
ABO#208(Gr.O)-less PRP	Negative	Positive	Negative
ABO#208(Gr.O)-Add Back	Positive weak	Positive	Negative

B. HLA-Typing: Extra Fine grade and Fine grade Ultra High Molecular Weight Polyethylene porous plastic (Porex Technologies Inc., Fairburn, GA) was cut into two-inch long×0.5 inch strips. Five microliters of antibody with demonstrated specificity to the HLA-A2 antigen (Lots AZ-18 or ALBQ14, Plasma Services, Scottsdale, AZ) were spotted approximately one inch from the end of the porous plastic strip. The positive control for this assay consisted of spotting 5 ul of Rabbit Anti-Thrombocyte Membrane (Dako, Lot 035) which binds all platelets regardless of HLA phenotype. The strips were dried at either 37° C. or 25° C. for 15 minutes. The dried strips were stored at 25° C. with desiccant capsules (Dricap Co.).

Prior to performance of the assay, absorbent pads (Schleicher and Schuell, #A300) were attached to one end of the porous plastic strip. Platelets were previously typed for HLA-A2 antigen by the microlymphocytotoxicity procedure (University of California San Diego Medical Center). Blood samples were collected in Ethylene Diamine tetraacetic acid (EDTA) anticoagulant. Four to five drops (100–125 ul) of whole blood were added to the sample application end of the porous plastic strip. This ensured a sufficient volume of blood to flow laterally past the antibody spotted region. Following a three minute incubation at 25° C., 16–20 drops of wash reagent were added to the sample application end. This caused the unbound cells to flow from the antibody spotted region to the absorbent pad area.

A positive reaction was characterized by a red dot at the site of antibody application. This results when the antibody affixed to the porous plastic (Anti-HLA-A2) recognizes the HLA-A2 antigen present on the platelet cell surface and binds the platelets. This subsequently forms a lattice network which serves to entrap the red blood cells in the test sample. A negative reaction is identified as a clear or white region at the antibody application site.

Whole blood samples from two donors, previously typed by lymphocytotoxicity as HLA-A2, produced positive reactions; one donor who was typed HLA-A2 negative gave a negative reaction. All three donors, regardless of HLA profile, gave positive reactions with the Anti-Thrombocyte positive control. The above assay provides a simple alternative to current methods to provide platelet type evaluation to crossmatch donor/receptor in transfusions.

Results

Donor ID	Anti-HLA-A2 status*	Positive Control	RBC Lateral Flow
JS	Negative	Positive	Negative
RWE	Positive	Positive	Positive
NA	Positive	Positive	Positive

*As determined by microlymphocytotoxicity testing, UCSD Medical Center.

EXAMPLE 6

Detection and Quantitative Measurement of Apolipoprotein B

Fine grade Ultra High Molecular Weight Polyethylene porous plastic (Porex Technologies Inc., Fairburn, GA) was cut into 1.5 inch long×1.5 inch blocks. The block was assembled between two polystyrene pieces slightly larger than the porous plastic block. Six wells or windows were punched out of the top polystyrene piece at a distance of approximately 0.25 inches from the center of the porous plastic block. Five microliters of either Sheep Anti-Human Apolipoprotein B (Boehringer Mannheim, Lot 10688829-07) or serially diluted Sheep Anti-Human Apolipoprotein B antibody were spotted into individual wells. The positive control for this assay consisted of spotting 5 ul of Rabbit Anti-Erythrocyte Membrane (Dako, Lot 015) into a fourth well. This antibody binds all erythrocytes regardless of ABO Rh blood type. A Negative Control well is not spotted with any antibody, but rather it serves to demonstrate the absence of non-specific reactions and the complete removal of unbound red blood cells form the test area. The spotted units were dried at 37° C. for 30 minutes. The dried units were stored at 25° C. with desiccant capsules (Dricap, Multiform Desiccants, Inc.).

Blood samples from two donors were collected in EDTA anticoagulant. Five drops (100–125 ul) of whole blood were added to the center sample application well. This ensured a sufficient volume of blood to flow laterally past the antibody spotted regions. Following a 1.5 minute incubation at 25° C., 26–30 drops (approximately 0.60–0.75 ml) of wash reagent were added to the center sample application well. This caused the unbound cells to flow from the antibody spotted region to the absorbent region of the porous plastic block.

Assay specificity was tested by preparation of packed red blood cells from aliquots of each test sample to remove the Apolipoprotein B serum components. The remaining packed red blood cells were washed with phosphate-buffered saline (PBS) and resuspended in varying amounts of Apolipoprotein B free serum (Scantibodies Laboratories, Lot 753A). These replacement

study samples were subsequently tested according to the procedure described above.

Comparative studies were performed with a commercially available RIA product. A positive reaction was characterized by a red dot at the site of antibody application. This results when the porous plastic affixed antibody recognizes and binds the Apolipoprotein B serum component. The resulting lattice network which is formed as a result of this binding serves to entrap the red blood cells in the test sample. A negative reaction is identified as a clear or white region at the antibody application site.

Results

Donor Test Sample	RIA Value mg/dl*	RBC Lateral Flow Titer**
KR-whole blood	58	1:8
100% replacement	20	1:2
50% replacement	40	1:4
KV-whole blood	60	1:8
100% replacement	10	Undiluted

*As determined by Ventrex Apo B RIA kit, Lot 0167

**Based on the highest dilution of Sheep Anti-Apolipoprotein B giving a positive reaction comparable to the positive control reaction.

The results show that the assay can be made semiquantitative by using varying concentrations (in this case serial dilutions) of the specific binding reagent in the indicator zone. Higher amounts of analyte are detected by more dilute antibody preparations.

The indicator zones can thus be graduated for detection and calibrated to obtain the semiquantitative measure of analyte. For example, as shown above, 1:8 dilution will detect 58–60 mg.dl apo B (and all higher concentrations); however, 1:4 dilution will detect 40 mg/dl, while the 1:8 dilution will not. Thus, the highest dilution at which reaction is detectable will give a measure of the concentration of analyte.

EXAMPLE 7

Assay for Group A Streptococcus

A. Fixation of Specific Binder to Indicator Zones

Three different approaches were developed for immobilizing antibody within the support matrix:

1. Antibody was concentrated by Amicon ultrafiltration or collodion bag, and applied directly to the intended indicator zone of a Porex porous plastic strip. The Porex was then dried.

2. Antibody was concentrated as above, biotinylated, and applied to the dried indicator zone containing affixed avidin, and then dried.

3. Polystyrene beads, adsorbed with antibody, were applied to the Porex to obtain an indicator zone in the form of a spot. The beads could also be sprayed onto the desired portion of the Porex, so that bars or lines could be used as indicator zones.

Details

A.1 In the first approach, a 10–20 ul spot of concentrated DEAE purified rabbit anti-Group A strep antibody at 10–30 mg/ml in 0.1 M Na_2CO_3 buffer, pH 9.5 was applied approximately ⅜" from the end of the Porex strip. The strip was dried for three hours at 37° C.

A.2 In the second approach, a 10–20 ul spot of avidin at 10 mg/ml in 0.1 M Na_2CO_3 buffer pH 9.5 was applied approximately ⅜" from the end of the Porex strip. The strip was dried for three hours at 37° C. A second spot of biotinylated rabbit anti-Group A strep antibody at 10–30 mg/ml was then applied to the avidin spot, and the strip again dried for three hours at 37° C.

A.3 In the third approach, polystyrene beads from Polysciences, Warrington, PA were used to adsorb polyclonal antihuman gonadotropin antibodies.

One volume of polystyrene bead suspension (0.2–1 u diameter, 2.5% solids) was added to two volumes of affinity purified polyclonal anti-hCG in 0.75 mg/ml in glycine buffered saline (0.1 M Glycine, 0.2 M NaCl, pH 8.2). The antibody bead suspension was mixed for 24 hours at 25° C., and then centrifuged in an Eppendorf model 5414 centrifuge for five minutes. The supernatant was discarded and the beads were suspended in blocking solution (0.1 M KPO_4 pH 7.2, 1% BSA, 0.02% NaN_3), mixed for three hours at 25° C., and the beads were again centrifuged as above, washed, mixed, and centrifuged twice with the blocking buffer. The beads were finally resuspended in the same buffer at a concentration of 1% solids based upon starting concentration.

A 20 ul spot of these antibody absorbed beads at 1% solids was spotted on to the Porex strip approximately ⅜" from the end of the strip. The beads were dried for three hours at 37° C.

B. Assay for Streptococcus A Using Enzyme Detection

A 500 ul suspension of nitrous acid extracted Group A streptococcus was prepared by treating the equivalent of 5×10^5 bacteria with 2 M nitrous acid for one minute, and neutralizing with 0.75 M NaOH, 0.5 M Tris. To this suspension was added 100 ul of a conjugate of rabbit anti-Group A streptococcus which had been partially purified over DEAE cellulose (DE52, Whatman) and conjugated to alkaline phosphatase. The mixture was applied dropwise to the end of a Porex strip. The strip contained an indicator zone to which was affixed antistreptococcus A. Fixation was as described in Section A above. The sample was allowed to flow laterally past the immobilized antibody (indicator zone) spot and into an absorbent pad at the end of the strip.

The strip was incubated for 2–3 minutes, and washed by applying 1 ml of borate-saline-TWEEN TM detergent wash buffer (2 ml/l TWEEN TM detergent 20; 0.477 g/l Na borate . 10 H_2O; 0.309 g/l boric acid; 0.2 g/l Na azide; 23.3 g/l NaCl). The buffer was added dropwise to the end of the strip to flow laterally past the indicator zone into the absorbent pad. The bound streptococcus A was detected by applying 0.5 ml of alkaline phosphatase chromogen (2.1 mg/ml 5-bromo-chloro3-indolylphosphate, p-toluidine salt, pH 10.1 in 26.7 g/l aqueous aminomethyl propanol) to the end of the strip to flow laterally past the indicator zone. After 1–2 minutes incubation, the results were read; blue color signifying positive response, no color as a negative response.

EXAMPLE 8

Assay for hCG

A solution of hCG was prepared as a 500 ul sample containing 50 mIU hCG in buffered saline or urine. To this was added 100 ul of a conjugate of monoclonal anti-hCG which had been partially purified over DEAE celluose and conjugated to alkaline phosphatase. The mixture was applied dropwise to the end of a Porex porous plastic strip containing an indicator zone with affixed anti-hCG antibody prepared as in Preparation A.3 of Example 7. The sample was allowed to flow laterally past the antibody absorbed bead spot (indicator

zone) and into an absorbent pad at the end of the strip. After incubating the strip for 2-3 minutes, the strip was washed by applying 1 ml of borate-saline-TWEEN TM detergent wash buffer dropwise to the end of the strip to flow laterally past the indicator zone into the absorbent pad. As above, 0.5 ml of alkaline phosphatase chromogen was applied to the end of the strip to flow laterally past the indicator zone. The results were read 1-2 minutes after incubation, blue color signifying positive response, no color signifying a negative response.

Industrial Application

This invention finds application in basic and clinical research medicine, immunology, and the like, in forensic science, and in the biochemical and biological industries generally.

We claim:

1. Apparatus for determining the presence or absence or the approximate amount of an analyte in a liquid sample which method comprises:

- a non-bibulous lateral flow membrane; wherein said membrane has on its surface;
- a liquid sample application zone defined to receive said liquid sample;
- at least one indicator zone spaced laterally apart from the application zone on said surface, wherein immobilized in said indicator zone is a member of a binding pair capable of binding said analyte or a derivative of said analyte; and
- wherein the lateral spacing of the application zone and the indicator zone is configured so as to cause the liquid sample introduced at the application zone to flow by non-bibulous lateral flow through the indicator zone;
- so as to permit the immobilized member to react with said analyte or derivative thereof so that the presence or absence or approximate amount of analyte is determined in the indicator zone.

2. The apparatus of claim 1 which further includes an absorption zone in lateral contact with said indicator zone so as to cause said liquid sample to flow through the indicator zone and into the absorption zone.

3. The apparatus of claim 2 wherein the absorption zone comprises a contiguous portion of the non-bibulous lateral flow membrane.

4. The apparatus of claim 2 wherein the absorption zone comprises a bibulous absorption body in contact with the non-bibulous lateral flow membrane.

5. The apparatus of claim 1 wherein the membrane is elongated in the application zone and the indicator zone are linearly spaced along the non-bibulous lateral flow membrane.

6. The apparatus of claim 5 which comprises a multiplicity of indicator zones linearly spaced along the non-bibulous lateral flow membrane.

7. The apparatus of claim 1 which comprises a multiplicity of indicator zones spaced radially around the liquid sample application zone.

8. The apparatus of claim 7 wherein the indicator zones are equidistant from the liquid sample application zone.

9. The apparatus of claim 1 wherein the member of the binding pair immobilized in the indicator zone is an antibody or fragment thereof.

10. The apparatus of claim 9 wherein the antibody or fragment thereof immobilized in the indicator zone is immunoreactive with a blood group antigen.

11. The apparatus of claim 1 wherein the member of the binding pair immobilized in the indicator zone is an antigen.

12. The apparatus of claim 1 which further contains a sample receiving pad in contact and on the top surface of the liquid sample application zone on the non-bibulous lateral flow membrane.

13. The apparatus of claim 12 wherein said sample receiving pad contains a reagent reactive with analyte to form a derivative of said analyte which derivative is reactive with the member of the binding pair that is immobilized in the indicator zone.

14. The apparatus of claim 13 wherein the reagent is an antibody or antigen or fragment thereof.

15. The apparatus of claim 1 which further comprises a breakable container of liquid reagent, said container positioned so as to release the reagent laterally through the non-bibulous lateral flow membrane and through the indicator zone without passing through the liquid sample application zone.

16. The apparatus of claim 15 which further includes a means to break said container.

17. An apparatus for measuring the presence or absence or approximate quantity of an analyte in a liquid sample which apparatus comprises, in combination:

- a non-bibulous lateral flow membrane;
- a housing for holding the membrane, said holding being so constructed as to form;
- means defining an application zone on the non-bibulous lateral flow membrane for receiving a liquid sample in said application zone;
- at least on indicator zone laterally apart from said application zone on said membrane to which is immobilized a member of a binding pair, said member being reactive with with analyte or a derivative or said analyte; and
- reading access means for permitting the indicator zone to be read, thus permitting measuring the presence or absence or approximate quantity of analyte by reading said indicator zone.

18. The apparatus of claim 17 which further comprises a bibulous absorbent body; and

- means for positioning a portion of the non-bibulous lateral flow membrane into contact with the absorbent body for permitting liquid to flow from the membrane to the absorbent body.

19. The apparatus of claim 17 further comprising a sample receiving pad on top of and contacting the liquid sample application zone.

20. The apparatus of claim 19 wherein said sample receiving pad contains a reagent capable of reacting with analyte to form a derivative of said analyte which is reactive with the member of the binding pair that is immobilized in the indicator zone.

21. The apparatus of claim 17 further comprising:

- at least one breakable container of liquid reagent,
- said container positioned to permit liquid to flow from the container, upon being broken in use, into the non-bibulous lateral flow membrane and through the indicator zone without passing through the liquid sample application zone.

22. A method for determining the presence or approximate amount of analyte in a sample comprising the steps of:

- placing the sample on the liquid sample application zone of a non-bibulous lateral flow membrane which comprises a liquid sample application zone and at least one indicator zone spaced apart from

said application zone laterally on the surface of said membrane, there being immobilized in said indicator zone a member of a binding pair capable of binding the analyte or a derivative thereof,

said sample being of sufficient quantity to cause sample liquid to flow laterally from the application zone through said indicator zone to permit the binding of analyte or derivative thereof with the member of the binding pair immobilized in the indicator zone; and

assessing the binding of the analyte or derivative thereof in the indicator zone, to determine the presence, absence or approximate amount of analyte.

23. The method of claim 22 wherein the member of the binding pair immobilized in the indicator zone is an antibody or fragment thereof and the analyte is an antigen which contains an epitope reactive with the antibody or fragment thereof.

24. The method of claim 23 wherein the antigen is a soluble antigen.

25. The method of claim 24 wherein the antigen is selected from the group consisting of, tissue-specific cell surface markers, tissue-shared cell surface markers, viral-associated cell surface markers, tumor-specific cell surface markers, bacterial polysaccharides, viral coat proteins, hormones, drugs, and antibodies.

26. The method of claim 24 wherein the antigen is selected from the group consisting of apolipoprotein B and apolipoprotein A1.

27. The method of claim 23 wherein the antigen is bound to the surface of a particle.

28. The method of claim 27 wherein the particle is a cell.

29. The method of claim 27 wherein the particle is selected from the group consisting of red blood cells, platelets, tumor cells, bacteria, viruses, and viral particles.

30. The method of claim 29 wherein the particle is a red blood cell.

31. The method of claim 22 wherein the derivative of the analyte is a conjugate of said analyte with a member complementary to the member of the binding pair immobilized in the indicator zone.

32. The method of claim 31 wherein the member conjugated to analyte is further conjugated to label.

33. The method of claim 31 wherein said member complementary to the binding member immobilized in the indicator zone is applied to the application zone along with the analyte so as to effect conjugation of said member to the analyte in the application zone.

34. A method to detect the formation of a specific binding pair complex in an indicator zone of a non-bibulous lateral flow membrane said membrane having a pore size of 1-250 microns and having on its surface a liquid sample application zone defined to receive said liquid sample and at least one indicator zone spaced laterally apart from the application zone; wherein immobilized in said indicator zone is a member of a binding pair bound to its a complementary member of said binding pair so as to form a complex,

which method comprises applying to the surface of said membrane detectable particles of sufficient size to be entrapped by said complex and causing said particles to flow laterally through said indicator zone, and

detecting the presence of the particles in the indicator zone as a means of detecting the formation of the specific binding pair complex.

35. The method of claim 34 wherein the particles are red blood cells.

36. The method of claim 34 wherein the particles are colored latex.

37. The method of claim 34 wherein said detectable particles are present in a sample providing an analyte, which analyte is a member of said complex.

38. The method of claim 37 wherein said sample is whole blood.

39. A method for determining the presence or absence or approximate amount of an analyte in a liquid sample comprising the steps of:

applying the sample to the liquid sample application zone of a non-bibulous lateral flow membrane having a pore size of 1-250 microns which membrane comprises said application zone and at least one indicator zone spaced laterally apart from said application zone on the surface of said membrane, there being immobilized in said indicator zone a member of a binding pair capable of binding the analyte or a derivative thereof,

said sample being of sufficient quantity to cause sample liquid to flow laterally from the application zone through said indicator zone; and

detecting the binding of the analyte or derivative thereof in the indicator zone;

wherein said detecting is effected by entrapping detectable particles in a complex formed by the binding of the analyte or derivative thereof with the member of the binding pair immobilized in the indicator zone; and

assessing the entrapment of the particles in the indicator zone to determine the presence or absence of approximate amount of analyte.

40. The method of claim 39 wherein the particles are added to the liquid sample before said sample is applied to the application zone.

41. The method of claim 39 wherein the particles are suspended in liquid and applied to the liquid sample application zone after the sample has been applied to said application zone.

42. The method of claim 39 wherein the particles are present in the sample.

43. The method of claim 39 wherein the particles are red blood cells.

44. The method of claim 39 wherein the particles are colored latex particles.

45. A method of type blood, which method comprises applying a sample of whole blood to the liquid sample application zone of a non-bibulous lateral flow membrane, wherein the membrane comprises:

said application zone defined to receive said whole blood sample, and

at least one indicator zone spaced laterally apart from the application zone, said indicator zone having immobilized therein an antibody or fragment thereof immunoreactive with a blood typing antigen,

said whole blood sample being of sufficient volume to permit the whole blood to flow through the indicator zone, and

applying sufficient clear liquid to the membrane at a position apart from the indicator zone so as to laterally flow through the indicator zone to re-

move unbound reed blood cells from said indicator zone and

detecting the presence or absence of red color in the indicator zone so as to determine the presence or absence of a blood typing antigen immunoreactive with the antibody or fragment thereof immobilized in the indicator zone.

46. The method of claim 45 wherein the membrane comprises an indicator zone in which is immobilized an antibody or fragment thereof having reactivity with group A antigen and an indicator zone in which is immobilized an antibody or fragment thereof having reactivity with group B antigen.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,943,522

DATED : July 24, 1990

INVENTOR(S) : Robert W. Eisinger, Mohammed H. Khalil, David H. Katz, Robert B. Sargeant

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 29, line 20, after the word "which" and before the word "comprises", please delete the word "method".

In column 29, line 22, after the word "surface", please delete ";" and substitute --:--.

Signed and Sealed this

Twenty-first Day of January, 1992

Attest:

HARRY F. MANBECK, JR.

Attesting Officer *Commissioner of Patents and Trademarks*

US005591645A

United States Patent [19]

Rosenstein

[11] **Patent Number: 5,591,645**

[45] **Date of Patent: Jan. 7, 1997**

[54] **SOLID PHASE CHROMATOGRAPHIC IMMUNOASSAY**

[75] Inventor: **Robert W. Rosenstein**, Ellicott City, Md.

[73] Assignee: **Becton, Dickinson & Co.**, Franklin Lakes, N.J.

[21] Appl. No.: **49,247**

[22] Filed: **Apr. 20, 1993**

Related U.S. Application Data

[63] Continuation of Ser. No. 818,000, Dec. 30, 1991, abandoned, which is a continuation of Ser. No. 31,023, Mar. 27, 1987, abandoned.

[51] **Int. Cl.**[6] **G01N 33/543**; G01N 33/544; G01N 33/538; G01N 33/558

[52] **U.S. Cl.** **436/514**; 436/518; 436/524; 436/528; 436/541; 436/810; 436/829; 435/7.1; 435/810; 435/805; 435/970; 435/287.7; 435/287.8; 435/287.9; 422/56; 422/58; 422/60

[58] **Field of Search** 435/7.92–7.95, 435/970, 310, 7.1, 805; 436/501, 514, 515, 518, 523, 524, 541, 810, 829, 528; 422/56, 58, 60, 57

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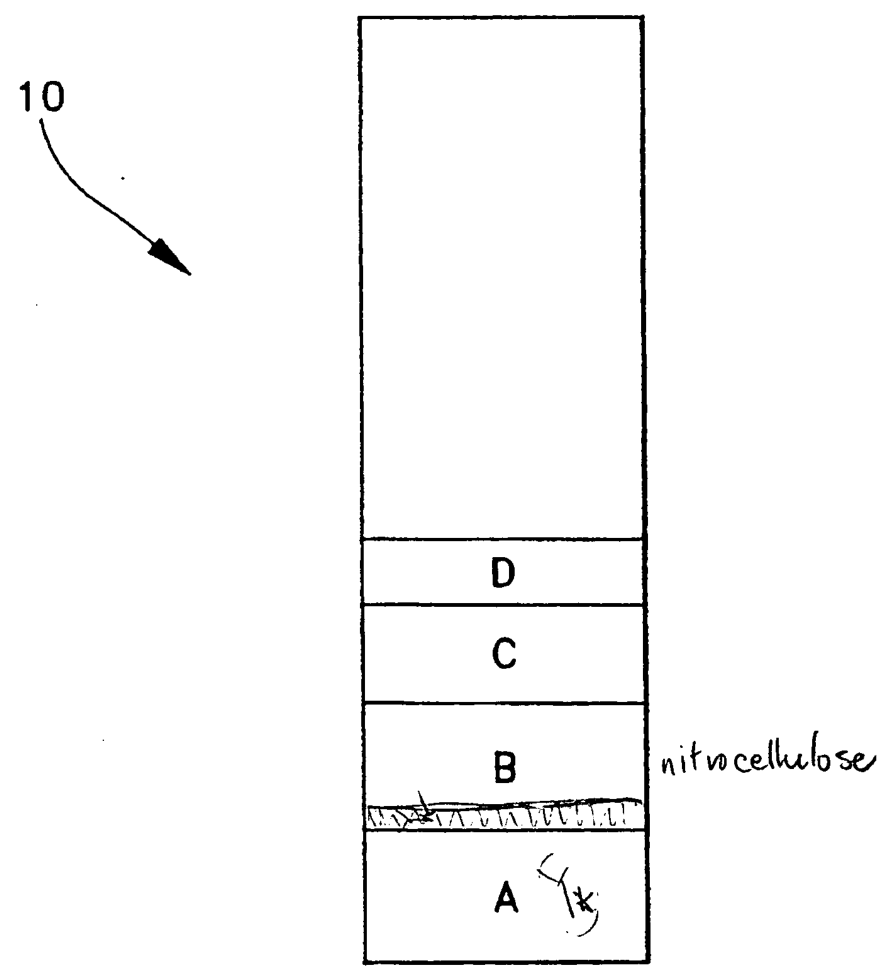
Primary Examiner—Carol A. Spiegel
Attorney, Agent, or Firm—Robert M. Hallenbeck

[57] **ABSTRACT**

A chromatographic test strip comprising a solid support having at least a first portion and a second portion with said portions being in the same plane so as to permit capillary flow communication with each other. The sample is added to the first portion. The first portion also may comprise a tracer portion having a tracer movably supported therein. The tracer consists of a visible particulate marker. In the second portion, a binder is immobilized. The test strip is useful in a variety of immunoassays.

23 Claims, 1 Drawing Sheet

FIG-1



SOLID PHASE CHROMATOGRAPHIC IMMUNOASSAY

This application is a continuation of U.S. Ser. No. 07/818,000, filed 30 Dec. 1991 (now abandoned), which is a continuation of U.S. Ser. No. 07/031,023, filed 27 Mar. 1987 (now abandoned).

BACKGROUND OF THE INVENTION

This invention relates to an assay for an analyte, and more particularly to a solid phase assay.

FIELD OF THE INVENTION

Assays for various analytes have been accomplished by a so-called solid phase assay. In a solid phase assay, a binder specific for at least the ligand to be determined (analyte) is supported on a solid support, whereby, in the assay it is not necessary to employ an additional agent for separating the bound and free phases formed in the assay.

In general, such solid supports have been in the form of tubes, solid particles, and in some cases, the solid phase has been in the form of a "dip-stick".

In a dip-stick solid phase assay, a binder may be supported the dip-stick with the dip-stick, containing the binder, being dipped into an assay solution containing the analyte, and in general, such solution further contains a tracer. The presence and/or amount of tracer on the dip-stick is then employed as a measure of analyte (either a qualitative or quantitative measure of analyte).

The present invention is directed to providing an improved solid phase assay for determining analyte, and more particularly to a solid phase assay.

SUMMARY OF THE INVENTION

In accordance with one aspect of the present invention, there is provided a solid support having a first portion and a second portion with the first and second portions being in capillary flow communication with each other whereby material flows by capillarity. The first and second portions are positioned on the solid support in a manner such that the first portion may be contacted with material, including any analyte, with material in said first portion being transported by capillarity from the first portion of the support to the second portion thereof.

sample receiving

The second portion of the solid support includes a binder which is a binder for at least the analyte, with the binder also being a binder for a tracer used in the assay, when the assay format is a so-called competitive assay format.

porous carrier

The solid support also includes a tracer, which is comprised of a ligand portion and a detectable label portion conjugated to the ligand portion of the tracer. In the case where the assay format is a so-called competitive assay format, the ligand portion of the tracer is bound by the binder contained in the second portion of the solid support. In the case where the assay format is a so-called sandwich assay format, the ligand portion of the tracer is bound by the analyte.

The tracer is supported on the solid support on a tracer portion of the solid support in a manner such that when wetted, the tracer is capable of being transported by capillarity to the second portion of the solid support, and thereafter, depending on the presence and/or absence of analyte and/or the amount of analyte, as hereinafter explained in more detail, to a third portion of the solid support.

= macroporous

The tracer portion of the solid support may be a separate portion of the solid support or may be the first portion of the solid support (the portion to which sample is added).

The binder which is supported on the second portion of the solid phase is supported in a manner such that the binder remains immobile and is not transported by capillarity to the third portion of the solid support.

The third portion of the solid support may be a portion for detecting tracer which has been transported by capillarity from the second portion to the third portion. The third portion may or may not include a substance supported thereon for detecting tracer. Alternatively, the third portion may function only to receive materials not bound in the second portion.

In accordance with the present invention, the amount of tracer which is immobilized in the second portion of the solid support by being bound either directly to the binder in the second portion (in a competitive assay format), or by being indirectly bound to the binder (tracer is bound to analyte which is bound to the binder in a sandwich assay format) is dependent upon the presence and/or amount of analyte in the sample. In a so-called sandwich assay format, the amount of tracer which is passed from the second portion to the third portion of the solid support by capillarity is indirectly proportional to the amount of analyte in the sample, and in the so-called competitive assay format, the amount of tracer which passes from the second portion to the third portion of the solid support, by capillarity, is directly proportional to the amount of analyte in the sample.

In a preferred embodiment of the present invention, the solid support and the various components are produced and employed in a manner for determining analyte by a competitive assay format, with the tracer being supported on the first portion of the solid support.

In a particularly preferred embodiment, as hereinafter explained in more detail, the detectable label portion of the tracer is comprised of a sac or lipid vesicle (often referred to as a liposome), which includes a detectable label.

In employing a preferred embodiment wherein the assay is a competitive assay, the tracer is supported on the solid support on the first portion thereof, and the first portion of the solid support is wetted with the sample containing analyte to be determined. Upon wetting of the solid support with the sample, both sample and tracer flow by capillarity into the second portion of the solid support which contains a binder specific for both the analyte and tracer, with the binder being immobilized on the second portion of the solid support. Depending upon the presence and/or amount of analyte in the sample portion, tracer becomes bound to the binder on the second portion of the solid support. The tracer which is not bound by the binder on the second portion, then flows by capillarity into the third portion of the solid support for detection and/or determination therein. If the assay format is to be a simple "yes or no" format (only determining whether or not analyte is present in the sample), then the binder supported on the second portion of the solid support is supported in an amount such that in the absence of a detectable amount of analyte in the sample, there is no detectable presence of tracer in the third portion of the solid support. As should be apparent, as the amount of analyte in the sample increases, the amount of tracer which is not bound to the binder in the second portion of the solid support increases, thereby increasing the amount of tracer present in the third portion of the solid support. Accordingly, a quantitative assay may be run by determining tracer which remains in the second portion of the solid support and/or

control

which flows by capillarity into the third portion of the solid support, and comparing such detected amount of tracer in the second and/or third portion with a "standard curve" to determine the amount of analyte in the sample. Thus, in an assay the determination of tracer and/or analyte may be either qualitative or quantitative.

In the sandwich assay format, tracer is preferably supported on a tracer portion of the solid support which is different from the first portion of the solid support. The ligand portion of the tracer is bound by the analyte, with the binder in the second portion of the solid support being specific for the analyte. The first portion of the solid support is contacted with the sample containing analyte, and the tracer portion of the solid support is wetted to cause both the tracer and analyte to flow by capillarity to the binder supported by the second portion of the support. The amount of tracer which becomes bound to analyte is directly proportional to the amount of analyte in the sample, and tracer bound to analyte, as well as any unbound tracer, flow by capillarity to the second portion of the solid support. In the second portion of the solid support, analyte becomes bound to immobilized binder specific for the analyte, with the unbound tracer (tracer not bound to analyte which is bound to the immobilized binder) flows by capillarity to the third portion of the solid support. The tracer on the third portion of the solid support may be detected as a measure of the presence and/or amount of analyte in the sample.

control

In a "yes or no" sandwich assay type format, the amount of tracer which is employed on the first portion of the solid support as well as the amount of binder on the second portion of the solid support are such that in the presence of a detectable amount of analyte, essentially no detectable tracer flows into the third portion of the solid support.

In a sandwich assay format, the amount of binder which is employed on the second portion of the solid support is an amount such that essentially all of the analyte which is suspected of being present in the sample is bound by the binder on the second portion.

The solid support which is employed in the assay is one which is capable of absorbing analyte from the sample, and which, when wetted, provides for flow of analyte and tracer by capillary attraction from the first portion, and through the second portion into the third portion of the solid support. In addition, the solid support is one which is capable of supporting tracer and the binder. As representative examples of suitable solid supports there may be mentioned: glass fiber, cellulose, nylon, crosslinked dextran, various chromatographic papers, nitrocellulose, etc. A particularly preferred material is nitrocellulose.

strip w/ 3 diff. portions

The solid support is preferably shaped in the form of a strip, with the first, second and third portions being arranged on the strip in the same plane in a manner such that material can flow by capillary attraction from the first zone and through the second zone to the third zone. Although the preferred shape is in the form of a strip, any other of a wide variety of shapes or forms may be employed as long as the shape and form permits separate portions for performing the various functions, as hereinabove described.

The tracer employed in the assay, as hereinabove indicated, is comprised of a ligand portion and a detectable label portion conjugated to the ligand portion. The detectable label of the detectable label portion may be any one of a wide variety of detectable labels; however, in accordance with a preferred embodiment, the detectable label is one which provides a color change in the second and/or third portion of the solid support, which is either a visible color

change, or one which requires an instrument to detect the change in color. In accordance with a preferred embodiment, the label which is employed provides a change in color in the second and/or third portion of the solid support which is visible without the use of an instrument. For example, such a change in color may be provided by employing an enzyme as the detectable label, and by providing a substrate for the enzyme in the third portion of the solid support, which substrate, when contacted with the enzyme, provides a visible detectable change in color. Alternatively, the detectable label may be the substrate, and the third portion of the solid support may be provided with the enzyme, whereby there is a detectable change in color in the third portion by contacting of the enzyme with the substrate label. As representative examples of other detectable labels, which may or may not require an instrument for detecting a color change, there may be mentioned various chromogens, such as fluorescent materials, absorbing, dyes, and the like. As hereinafter indicated in a competitive assay, a preferred label portion is a vesicle, which includes a detectable marker, with the detectable marker being one which is visible.

The ligand portion of the tracer is dependent upon the assay format. If the assay is a competitive assay, then the ligand portion of the tracer is either the analyte or an appropriate analogue thereof. An appropriate analogue means that the analogue of the ligand is also specifically bound by the binder for the analyte. If the assay format is a sandwich type of assay, then the ligand portion of the tracer is a ligand which is specifically bound by the analyte or by an antibody which is specifically bound by the analyte.

The binder which is employed in the assay is one which at least binds the analyte. As hereinabove indicated, if the assay format is a competitive type of assay format, then the binder also binds the ligand portion of the tracer.

As generally known in the art, if the analyte is an antigen or a hapten, then the binder may be either a naturally occuring binder or an antibody which is specific for the analyte (either a polyclonal and/or monoclonal antibody). If the analyte is an antibody, the binder may be either an antigen specific for the antibody or an antibody which specifically binds the antibody analyte.

antibodies

The binder may be supported on the solid support in a manner which immobilizes the binder; e.g., adsorption, covalent coupling, etc. The procedures for immobilizing binders on a solid support are generally known in the art.

The tracer, when supported on the first portion of the solid support, is supported in a manner such that when the first portion is wetted the tracer flows by capillary action. Thus, for example, the tracer may be absorbed on the first portion of the support.

In accordance with a particularly preferred embodiment of the present invention, in a competitive assay, the tracer is comprised of a ligand conjugated to a vesicle, which vesicle contains a detectable marker, with the tracer being supported on the solid support. Applicant has found that it is possible to support such a tracer on a solid support of the type hereinabove described, and that such tracer will flow by capillarity when the solid support is wetted with a sample containing or suspected of containing an analyte.

The lipid vesicles (liposomes) which are employed may be prepared from a wide variety of lipids, including phospholipids, glycol lipids, and as representative examples there may be mentioned lecithin, spingomyelin, dipalmitoyl lecithin, distearoylphosphatidylcholine, etc. The amphiphilic lipids employed for producing liposomes generally have a hydrophilic group, such as a phosphato, carboxylic, sulfato,

or amino group, and a hydrophobic group, such as saturated and unsaturated aliphatic hydrocarbons, and aliphatic hydrocarbon groups substituted by one or more aromatic or cycloaliphatic groups. The wall forming compounds for producing the liposomes may further include a steroid component such as cholesterol, cholestanol, and the like. The compounds for producing liposomes are generally known in the art, and no further details in this respect are deemed necessary for a complete understanding of the present invention.

The liposomes may be produced by procedures generally available in the art. For example, liposomes may be produced by a reverse phase evaporation technique wherein the compound or compounds used in producing liposomes are initially dissolved in an organic phase, followed by addition of an aqueous phase and forming of a homogeneous emulsion. After forming the emulsion, the organic solvent is evaporated to form a gel like material, and such gel may be converted to a liposome by agitation or dispersion in an aqueous media.

Procedures for producing liposomes are described, for example, in U.S. Pat. No. 4,241,046; U.S. Pat. No. 4,342,828 and PCT International Publication No. WO 80-01515.

If a material is to be encapsulated in the liposome, such material may be encapsulated in the liposome by including the material in the aqueous solution in which the liposome is formed. Alternatively, the material may be encapsulated into a previously formed empty liposome (without material to be encapsulated) by the procedure described in U.S. Pat. No. 4,539,376.

The liposomes may also be produced by the procedures disclosed in U.S. Pat. No. 4,522,803.

The material which is entrapped or encapsulated within the liposome (the material is within the aqueous compartment or within the membrane bilayer of the liposome) is a detectable marker, such as dyes, radiolabels, fluorescent materials, chemiluminescent materials, electron spin resonance materials, and the like; substrates for detectable markers; and the like. Alternatively, the liposome may be derivatized with a detectable marker, rather than entrapping a marker in the liposome.

The liposome is derivatized with a ligand for producing a tracer. The liposome may be derivatized with a ligand by procedures known in the art, such as covalent coupling, derivatization or activation, etc. In derivatizing the liposomes with a ligand, a compound or compounds used in forming the liposome may be derivatized with the ligand, prior to forming the liposome, or alternatively, the liposome may be derivatized with the ligand, subsequent to forming of the liposome. Procedures for derivatizing liposomes with ligands, and suitable coupling agents, and the like for preparing derivatized liposomes are known in the art, and no further details in this respect are deemed necessary for a complete understanding of the present invention.

In employing a preferred tracer in which the detectable marker portion thereof is comprised of liposome including a detectable marker for use in a competitive assay, the assay may be accomplished as hereinabove described with general reference to a variety of tracers, except that the tracer includes a liposome as the detectable marker portion of the tracer.

In a particularly preferred embodiment, the tracer used in the assay is a ligand conjugated to a particulate label which is visible. The term "visible" as used herein means that the label can be seen without the use of instrumentation; i.e., with the naked eye. The particulate label is may be a metal or alloy (e.g. colloidal gold) or a sac in particular a liposome containing a visible dye. The marker preferably included in the sac is a dye or some other material which is visible, without lysing of the sacs.

gol 50/5

The tracer comprised of ligand and particulate label may also be produced by labeling the ligand with an aqueous dispersion of a hydrophobic dye or pigment, or of polymer nuclei coated with such a dye or pigment. Such labels are described in more detail in U.S. Pat. No. 4,373,932, which issued on Feb. 15, 1983. The tracers produced in accordance with such patent may also be employed as tracers in the present invention.

As indicated in the aforesaid patent, the colored organic compounds which are used as labels are in the form of a hydrophobic sol, which hydrophobic organic dyes or pigments are insoluble in water or soluble only to a very limited extent.

The visible particulate label may be visible polymer particles, such as colored polystyrene particles, preferably of spherical shape. polystyrene particles

As representative examples of other particulate labels which may be employed in producing a tracer for use in the assay of the present invention, in which the tracer would be visible, there may be mentioned; ferritin, phycoerythrins or other phycobili-proteins; precipitated or insoluble metals or alloys; fungal, algal, or bacterial pigments or derivatives such as bacterial chlorophylls; plant materials or derivative metal sols and the like. In such an embodiment, at least the portion of the product which includes the binder is formed of a material having a surface area capable of supporting the binder thereon in an amount such that tracer bound in such portion is visible. In general, the surface area is capable of supporting the binder in a concentration of at least 1 ug/cm^2, and most generally in a concentration of at least 10 ug/cm^2. A particularly preferred material is nitro-cellulose. Such materials and tracers are described in U.S. Pat. No. 4,703,017, which is hereby incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWING

FIG. **1** is a schematic representation of a chromatographic test strip wherein "A" represents the first portion of the test strip, "B" represents the second portion of the test strip and "C" and "D" represent a third portion of the test strip.

Referring to FIG. **1**, there is shown a strip **10** including a first portion A on which a tracer is supported; a second portion B on which a binder is supported and a third portion D in which tracer may be determined. As particularly shown, a portion C is between portions B and D to provide spacing between portions B and D, whereby the portion for determining tracer is separated by a distance from the portion containing binder.

In a competitive assay format, employing an enzyme as a detectable label, portion A would contain ligand labeled with enzyme, with the ligand portion being the analyte or appropriate analogue thereof; portion B would contain a binder specific for the analyte and the ligand portion of the tracer; and portion D would contain a substrate for the enzyme which interacts with the enzyme to provide a change in color.

In use, portion A of the strip **10** would be contacted with a sample containing analyte, whereby portion A would be wet with the sample. The tracer in portion A, as well as, sample would be transported by capillarity to portion B, where tracer and analyte compete for binding sites on the binder. Unbound tracer and unbound analyte move by cap-

illarity through portion C to portion D where any tracer interacts with the substrate in portion D to provide a change in color. As hereinabove indicated, the assay may be a "yes-no" assay or a quantitative assay and detection of tracer in portion D is dependent upon the assay employed.

In the case where the tracer has a detectable label which does not require an additional substance for determination thereof, the portion D would not require an additional substance, i.e., portion D would also be blank. Thus, for example if the tracer included a liposome having a dye as a detectable label, then tracer may be determined without supporting an additional substance on portion D. Alternatively, if for example, it was required to release detectable lable from the liposome, portion D could contain a suitable lysing agent, such as an enzyme or detergent which lyses liposomes to release label from the liposome in portion D for detection of tracer.

In addition, it is also possible to determine tracer in portion C, with or without determining tracer in portion D. For example, a substrate could be added to portion C in the case where the label is an enzyme.

The product may be used as a dip stick. Alternatively, a sample may be applied to portion A. Accordingly, the product may be used in either a horizontal or vertical orientation.

The invention is applicable to detecting and/or measuring a wide variety of analytes, such as: drugs, including therapeutic drugs and drugs of abuse; hormones, vitamins, proteins, including antibodies of all classes, peptides; steroids; bacteria; fungi; viruses; parasites; components or products of bacteria, fungi, viruses, or parasites; allergens of all types; products or components of normal or malignant cells; etc. As particular examples, there may be mentioned T_4; T_3; digoxin; hCG; insulin; theophylline; leutinizing hormone; organisms causing or associated with various disease states, such as *Streptococcus pyogenes* (group A), Herpes Simplex I and II, cytomegalovirus, chlamydia, rubella antibody, etc.

analytes

The invention will be further described with reference to the following example:

EXAMPLE

Dipsticks were constructed by first coating 0.5×8 cm strips of polystyrene with Scotch® #969 adhesive transfer tape (3M, St. Paul Minn. 55144). Zone B, consisting of a 0.5×0.5 cm square of 5 um-pore nitrocellulose (S&S, Keene, N.H.) was spotted with 3 ul of affinity purified rabbit anti-Group A *Streptococcus* antigen and then blocked with 3% bovine serum albumin. After drying, it was applied to the taped side of the dipstick, approximately 1 cm from the bottom of the stick. A strip of filter paper 0.5×6.5 cm. (Whatman 3 mm) was applied just above and touching the nitrocellulose, at the positions indicated by zones C and D. Zone A, consisting of dry SEPHADEX G50 fine grade bead-formed gel of cross-linked dextran (Pharmacia) was then applied.

label zone

DETAILED DESCRIPTION

Detector liposomes packed with sulfo-rhodamine dye were prepared by the method outlined in O'Connell et al. (Clin. Chem. 31:1424 [1985]). They were covalently coupled to affinity purified rabbit anti-Group A *Streptococcus* antigen.

The detector liposomes were spotted (2 ul) onto Zone A, 0.5 cm from the bottom and air dried. The liposomes are in a 0.05M Tris buffer, pH 6.8, containing 2% glycerol, 0.05% dimethyl sulfoxide, 20 mM EDTA.

Group A *Streptococcus* organisms were harvested from culture plates, washed with saline (0.9% NaCl), and adjusted to 1×10^9 organisms/ml. An aliquot (0.1 ml) containing 1×10^8 organisms was subjected to the micro nitrous acid extraction method for exposing the Group A carbohydrate antigen. This method consists of mixing 0.3 ml of 0.1M HCl with 40 ul of 4M $NaNO_2$, adding this to the *Streptococcus* organisms and, after 3 minutes, neturalizing with 40 ul of 1M Tris base. To faciliate the extraction and the dipstick assay, the HCl and the subsequent diluting fluid contain 0.1% Tween-20 non-ionic detergent.

Using the extracted antigen, a dilution series was prepared ranging from 8×10^6 organisms/ml to 1.25×10^5 organisms/ml. Aliquots of these dilutions (0.5 ml) were placed in 12×75 mm test tubes and a dipstick placed into the fluid in each test tube. As the fluid containing extracted antigen wicks up the stick, it carries the liposome detector past the spot of capture antibody. In the presence of antigen, which binds to the capture antibody spot, some of the liposomes also bind, resulting in the appearance of a red spot in zone B. The remainder of the liposomes and antigen solution pass into zone D.

The assay can be "read" by observing the lowest concentration of organisms resulting in a red spot in zone B. The results of this example are given in the following table and indicate the an end point of 5×10^5 organisms/ml, close to the sensitivity required for a direct throat swab diagnostic for Group A *Streptococcus* pharyngitis.

Group A Strep Antigen (organisms/ml) × 10^{-5}							
80	40	20	10	5	2.5	1.25	0
+	+	+	+	+	−	−	−

(+) = positive indication of antigen (red spot)
(−) = negative indication of antigen (red spot)

The present invention is advantageous in that there is provided a product and process which may be easily employed for accomplishing an assay. The product and process do not require the addition of tracer in that tracer is included in the product. In addition, the product and process are capable of providing for a rapid assay.

These and other advantages should be apparent to those skilled in the art from the teachings herein.

Numerous modifications and variations of the present invention are possible in light of the above teachings; therefore, the invention may be practiced otherwise than as particularly described.

What is claimed is:

1. A test strip for determining the presence of an analyte in a liquid sample comprising a solid support, said solid support comprising at least a first portion and a second portion, said portions being in the same plane so as to permit capillary flow communication with each other;

said first portion being the site for application of the liquid sample and further comprising a tracer site, said tracer site consisting of a tracer movably supported therein wherein said tracer comprises a ligand, which specifically binds to the analyte, conjugated to a visible particulate marker; and

said second portion being the site for visually determining the presence of the visible particulate marker, said second portion consisting of a binder immobilized therein which specifically binds to the analyte.

2. The test strip of claim 1 wherein the solid support comprises nitrocellulose.

3. The test strip of claim 1 wherein the visible particulate marker is selected from the group consisting of colloidal metals, colored liposomes, colored polymeric beads and polymerized dye molecules.

4. The test strip of claim 3 wherein the visible particulate marker is a colored liposome.

5. The test strip of claim 3 wherein the visible particulate marker is a colored polymeric bead.

6. The test strip of claim 1 wherein the analyte is an antigen and the ligand and the binder are antibodies thereto.

7. The test strip of claim 1 wherein the ligand and the binder are antigens or analogs thereof and the analyte is an antibody thereto.

8. The test strip of claim 1 wherein the first portion and the tracer portion are spatially separate from each other with the first portion being upstream of the tracer portion.

9. A no-wash, one-step method for determining the presence of an analyte in a liquid sample consisting of the steps of:

a) adding a liquid sample to the first portion of the test strip of claim 1;

b) allowing sufficient time for the liquid sample to flow to the second portion of the test strip; and

c) determining the presence of the analyte in the liquid sample by visual inspection of the second portion for the visible particulate marker wherein the presence of the analyte is indicated by the presence of the visible particulate marker.

10. The method of claim 9 wherein the liquid sample is added to the test strip by immersing the first portion into the liquid sample.

11. A test strip for determining the amount of an analyte in a liquid sample comprising a solid support, said solid support comprising at least a first portion and a second portion, said portions being in the same plane so as to permit capillary flow communication with each other;

said first portion being the site for application of the liquid sample and further comprising a tracer site, said tracer site consisting of a tracer movably supported therein wherein said tracer comprises a ligand, which is the analyte or an analog thereof, conjugated to a visible particulate marker; and

said second portion being the site for visually determining the amount of the visible particulate marker, said second portion consisting of a binder immobilized therein which specifically binds to the ligand.

12. The test strip of claim 11 which consists of a third portion in the same plane as the first and the second portions, all of said portions being in capillary flow communication with each other, and said third portion being an additional site for visually determining the amount of visible particulate marker bound therein.

13. The test strip of claim 11 wherein the visible particulate marker is selected from the group consisting of colloidal metals, colored liposomes, colored polymeric beads and polymerized dye molecules.

14. The test strip of claim 13 wherein the visible particulate marker is a colored liposome.

15. The test strip of claim 13 wherein the visible particulate marker is a colored polymeric bead.

16. The test strip of claim 11 wherein the analyte is an antigen.

17. The test strip of claim 11 wherein the analyte is an antibody.

18. The test strip of claim 11 wherein the first portion and the tracer portion are spatially separate from each other with the first portion being upstream of the tracer portion.

19. A no-wash, one-step method for determining the presence of an analyte in a liquid sample consisting of the steps of:

a) adding a liquid sample to the first portion of the test strip of claim 11;

b) allowing sufficient time for the liquid sample to flow to the second portion of the test strip; and

c) determining the presence of the analyte in the liquid sample by visual inspection of the second portion for the visible particulate marker wherein the presence of the analyte is indicated by the absence of the visible particulate marker.

20. A no-wash, one-step method for determining the amount of an analyte in a liquid sample consisting of the steps of:

a) adding a liquid sample to the first portion of the test strip of claim 12;

b) allowing sufficient time for the liquid sample to flow to the second portion and the third portions of the test strip; and

c) determining the amount of the analyte present in the liquid sample by visual inspection of the second portion and the third portion for the amount of the visible particulate marker bound in each portion wherein the presence of the analyte is indicated by the absence of the visible particulate marker.

21. The method of claim 19 wherein the liquid sample is added to the test strip by immersing the first portion into the liquid sample.

22. The method of claim 20 wherein the liquid sample is added to the test strip by immersing the first portion into the liquid sample.

23. The test strip of claim 11 wherein the solid support comprises nitrocellulose.

* * * * *

United States Patent [19]

Friesen et al.

[11] Patent Number: 4,861,711

[45] Date of Patent: Aug. 29, 1989

[54] SHEET-LIKE DIAGNOSTIC DEVICE

[75] Inventors: Heinz-Jürgen Friesen; Gerd Grenner, both of Marburg; Hans-Erwin Pauly, Dautphetal; Helmut Kohl; Klaus Habenstein, both of Wetter; Joseph Stärk, Ebsdorfergrund, all of Fed. Rep. of Germany

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[22] Filed: Dec. 13, 1985

[30] Foreign Application Priority Data

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[51] Int. Cl.[4] G01N 31/00

[52] U.S. Cl. 436/7

[58] Field of Search 436/514, 810; 435/7, 435/805, 810; 422/56, 169, 61, 101

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Primary Examiner—Christine M. Nucker

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[57] ABSTRACT

A solid diagnostic device for the quantitative determination of substances of biological affinity in biological fluids is described. A process is also described in which the biological fluid is brought into contact with a specific functional sector of the device, the fluid migrates through several functional sectors situated beside one another and containing suitable reagent components, and one or more substances of biological affinity are detected in such functional sectors which contain, for each substance to be detected, at least one combination partner of biological affinity, attached to a solid phase.

34 Claims, 2 Drawing Sheets

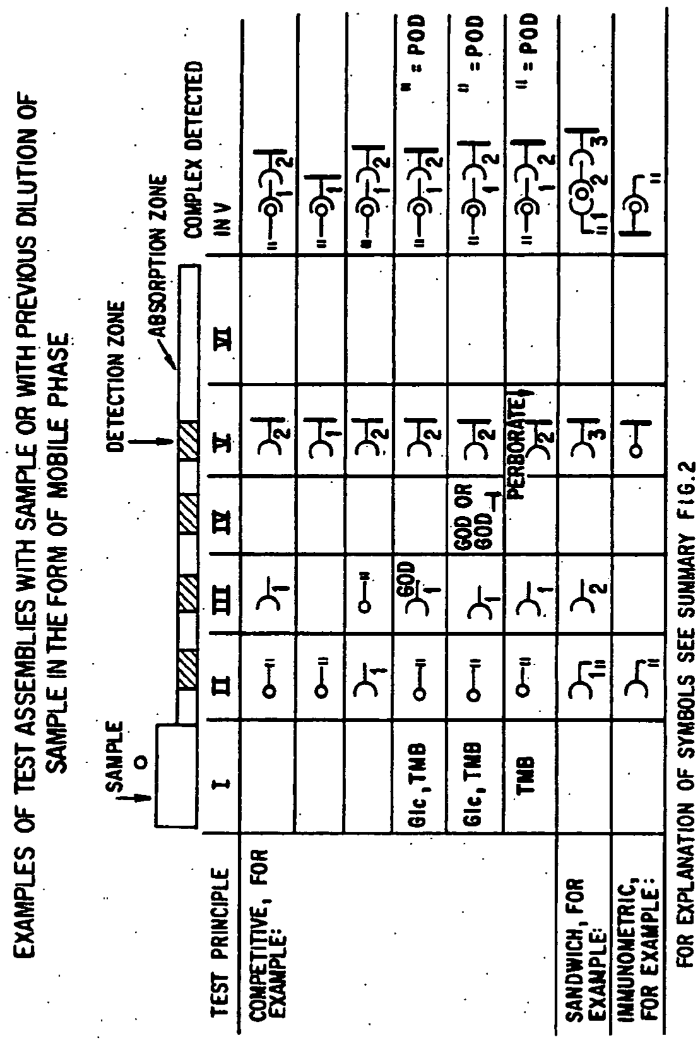
EXAMPLES OF TEST ASSEMBLIES WITH SAMPLE OR WITH PREVIOUS DILUTION OF SAMPLE IN THE FORM OF MOBILE PHASE
SAMPLE
DETECTION ZONE
ABSORPTION ZONE
TEST PRINCIPLE
I
II
III
IV
V
VI
COMPLEX DETECTED IN V
COMPETITIVE, FOR EXAMPLE:
Glc, TMB
Glc, TMB
TMB
GOD
GOD OR GOD
PERBORATE
" = POD
" = POD
" = POD
SANDWICH, FOR EXAMPLE:
IMMUNOMETRIC, FOR EXAMPLE:
FOR EXPLANATION OF SYMBOLS SEE SUMMARY FIG.2

FIG. 1

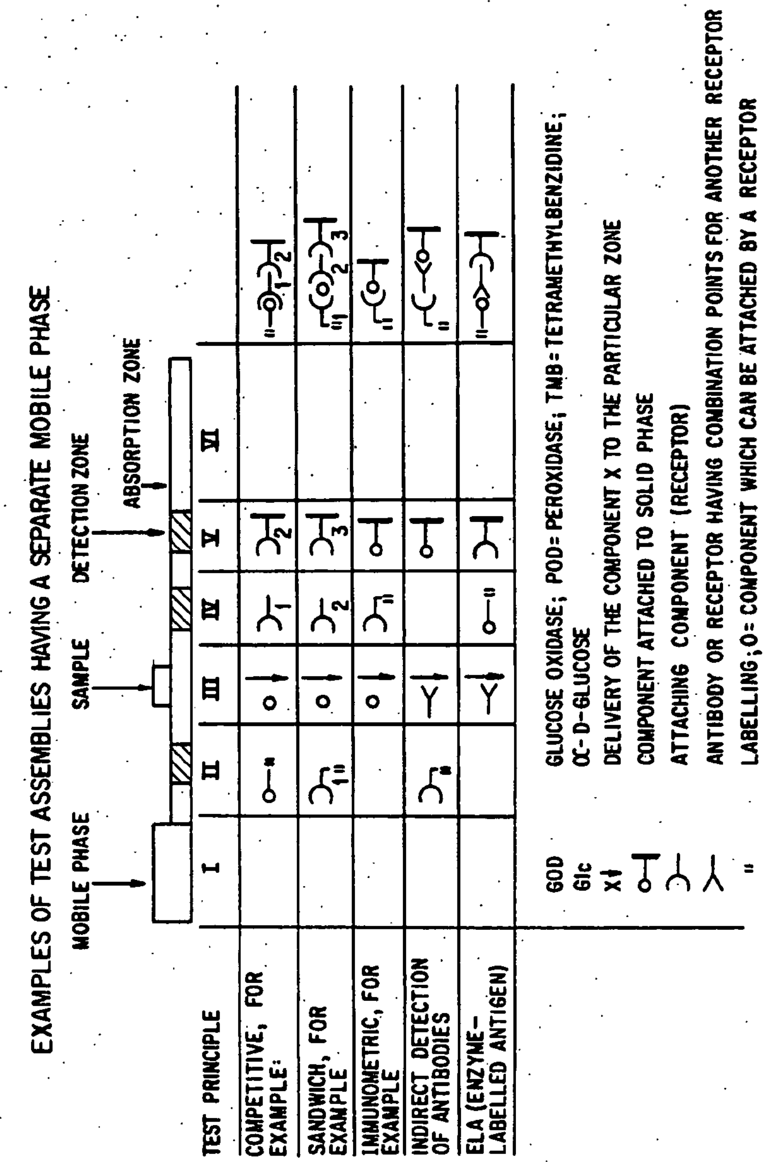
EXAMPLES OF TEST ASSEMBLIES HAVING A SEPARATE MOBILE PHASE
MOBILE PHASE
SAMPLE
DETECTION ZONE
ABSORPTION ZONE
TEST PRINCIPLE
I
II
III
IV
V
VI
COMPETITIVE, FOR EXAMPLE:
SANDWICH, FOR EXAMPLE
IMMUNOMETRIC, FOR EXAMPLE
INDIRECT DETECTION OF ANTIBODIES
ELA (ENZYME-LABELLED ANTIGEN)
GOD GLUCOSE OXIDASE; POD= PEROXIDASE; TMB= TETRAMETHYLBENZIDINE;
Glc α-D-GLUCOSE
DELIVERY OF THE COMPONENT X TO THE PARTICULAR ZONE
COMPONENT ATTACHED TO SOLID PHASE
ATTACHING COMPONENT (RECEPTOR)
ANTIBODY OR RECEPTOR HAVING COMBINATION POINTS FOR ANOTHER RECEPTOR
LABELLING; O= COMPONENT WHICH CAN BE ATTACHED BY A RECEPTOR

FIG. 2

SHEET-LIKE DIAGNOSTIC DEVICE

The invention relates to a solid diagnostic device which comprises several functional sectors and is used for the detection and quantitative determination of substances or analytes in biological fluids. The invention also relates to a process using this device in which, after the device has come into contact with the fluid, the analytes react with specific combination partners having biological affinity and are detected by means of labelling reagents.

In methods of diagnosis, the ability to identify and estimate specific compounds has made it possible to monitor the administration of medicaments, the quantification of physiologically active compounds or secondary products thereof and the diagnosis of infections. In this respect, the immunoassay methods (RIA, ELSIA and the agglutination test) are of particular importance. The specific combination reactions utilized in the tests are not limited to immunological interactions, such as antigen-antibody or hapten-antibody interactions, but also utilize interactions having biological affinity, such as lectin-sugar or active compound-receptor.

Although the existing tests are sensitive and specific, they do not constitute convenient application forms, because of the long duration of the test (in most cases several hours or even days) and the frequent test steps, such as immune reaction, washing steps and enzymatic reaction. The long test times are not compatible with use in emergency methods of diagnosis.

Integrated dry chemical test elements, such as are described in the present invention, simplify the performance of the tests and shorten the test times.

No sheet-like test element, in which all the components of the immune reaction of a heterogeneous immunoassay using solid phase detection, and the functional performance and the "bound-free" separation, are integrated has been described so far.

Whereas in the test strip assembly the immune reaction steps and the separation of bound and free phases are operated in the heterogeneous test by directed streams of liquid, in test element assemblies operating by means of thin layers laminated over one another (film technology), processes controlled by diffusion and directed by the concentration gradient are possible driving forces. A fluorescence labelling is used in German Offenlegungsschrift No. 3,329,728 (Japanese Patent No. P144,341/82) and EP A No. 0,097,952 (Japanese Patent No. 114,359/82). The labelling has a low molecular weight and hence promotes processes controlled by diffusion. However, the test has to be carried out at an elevated temperature. In the first of these two cases both the free phase and also the bound phase are evaluated. In film technology the absorption of solvent is effected either by hydrating swellable components or by filling capillary cavities. In the case of assemblies having layers laminated over one another only the top layer and the bottom layer are accessible to detection without major difficulties.

After the reaction steps have taken place it is difficult to react reagents with components in intermediately placed layers. In the test strip assembly having zones situated one behind another, such as is used in the present invention, in principle each zone is readily accessible, both from above and also from below, for a determination and also for the addition of reagents which may perhaps be required.

The invention relates to a sheet-like diagnostic device which contains all the reagent components and which contains not only all the components required for the functional sequence, but also the functional sequences themselves in an integrated form, and by means of which it is possible to detect an analyte having properties of biological affinity, in such a way that a solution of the analyte is brought into contact with a functional region of the device designed for this purpose, and the analyte as detected via a signal-producing system in a single functional region, a solid phase zone.

A second analyte, or further analytes, as constituents of the same solution can be detected at the same time by means of the device, if these analytes possess properties of biological affinity different from the first analyte. They are also detected in the same manner as the first analyte in a single functional region, a solid phase zone appropriate for them. The functional regions for the detection of the second or further analytes are situated on the sheet-like device in front of or behind the functional region for the detection of the first analyte. The device can also contain several solid phase zones which are appropriate for an analyte and different measurement ranges of this analyte. The device contains all reactants and reagents in a dehydrated form.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a summary table illustrating test assemblies according to certain preferred embodiments of the present invention; and

FIG. 2 is a summary table illustrating test assemblies according to certain preferred embodiments of the present invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

The sheet-like diagnostic device comprises one or several strips, arranged behind one another, of material which have a capacity for absorbing aqueous solutions. The strips are fixed on a solid support. They contain the reagent components required for the particular diagnostic agent and thus become functional sectors or functional regions. The functional sector situated at one end of the strip-shaped device (solvent application zone) is brought into contact with the analyte solution by being dipped into the latter or by the application of the latter. The solution migrates through all the functional regions. The absorptive capacity of the supporting materials of which the strips are composed causes a flow of liquid which stops at the other end of the strip-shaped device. The analyte can also be applied in the middle region of the device, and a flow of liquid from one end of the device to the other can then be induced.

The sample does not have to be applied directly to the chromatographing section of the device. It can also be applied to an absorptive material which is situated on the device and has the function of removing blood cells from the sample. After being filtered the sample then reaches the device. In the course of this filtration process the addition of reagents can be effected at the same time by dissolving the latter out of components present in the filter in a dry state. Interfering factors can be eliminated from the solution by means of such components. Thus, for instance, the ascorbic acid present in a sample, which interferes in the use of oxidases and peroxidases as labelling agents, can be rendered harmless by means of a suitable oxidizing agent. Furthermore, the filter can also have the function of an adsor-

bent which removes interfering factors from the sample by adsorption. The filtration, adsorption and reagent admixing function for conditioning the sample for the test can also be taken over by the mobile phase application zone or a zone situated behind the latter.

The distribution of the solvent in the individual functional regions depends on the adsorptive capacity and the dimensions of the materials used.

The solvent application zone can have the function of a volume metering element, as described in German Patent Nos. 3,043,608 and 2,332,760, and U.S. Pat. Nos. 3,464,560, 3,600,306, 3,667,607, 3,902,847, 4,144,306 and 4,258,001. It can contain, in dry form, the various reagents required for the function of the test element. The solvent application zone can be a piece of fabric paper which is located at one end of the test element and which becomes completely saturated with a definite volume of liquid merely by being dipped into a solution, for example a solution of the sample, or by being briefly flushed with tap water, and then releases the liquid to the succeeding zones more slowly and in a controlled manner. The solvent application zone has dimensions such that it takes up sufficient liquid to permit the latter to migrate to the other end of the device, the end of the absorption zone.

Between the solvent application zone and the absorption zone there are located the functional regions in which are contained reaction components for the performance of the test and in which all the reaction stages of the performance of the test take place. Part of the reaction components for the performance of the test can also be housed in the sample application zone. The absorption zone has the function of absorbing excess and freely mobile reagent components and reaction products of the single-producing system.

The absorbent supporting materials in the form of one or more strips, as constituents of the various functional regions, can, according to choice, be composed of cellulose, of chemical derivatives of cellulose or of plastics having a porous or fibrous structure and adequately hydrophilic properties, or of particles such as cellulose or silica gel embedded in a synthetic membrane, and also of natural products which are hydrophilc but have been rendered insoluble in water. A combination of strips composed of different materials can be used. Suitable absorbent materials are selected on the basis of the requirements set for the particular diagnostic device.

Reactants with immunological binding properties such as antigens, haptens or antibodies are incorporated in various embodiments of the device. In the event that glycoproteins or oligosaccharides which attach themselves to lectins are to be detected, one reactant having biological affinity can be the specific lectin, while the second reactant having immunological affinity can be an antibody which is directed against a point of attachment on the analyte other than that of the lectin. In the event that microbial active compounds are to be detected, One combination partner can be the receptor substance for the active compound, while the second combination partner can be an antibody which is directed against another point of attachment on the active compound.

One combination partner having biological affinity becomes attached during the progress of the reaction, or has already been attached to the supporting material in the functional region designed for the detection of the analyte (solid phase zone). It is also called the solid phase combination partner. The other combination partner(s) are present in the supporting materials. They are provided with a labelling.

Amongst the various known possibilities of labelling, enzyme labelling is preferred. It requires chromogenic substrate systems or substrate systems which produce fluorescence or chemiluminescence. Chemiluminescence labelling represents a further example of a labelling which is only measured after the addition of a reagent. It is possible to measure either the chemiluminescence itself or a fluorescence excited by the latter. In most cases fluorescence labelling is measured without the addition of a reagent being required. However, as in the use of certain rare earth chelates, it can also be desirable to produce the fluorophore to be measured only as the result of adding a reagent, or to add a second fluorophore which becomes excited by the first or which excites the first fluorophore. The fluorescence can be measured at one point, as a function of time or as fluorescence polarization.

A reagent required for detection can be induced to react with the immune complex to be detected in various ways, after the separation stage. Part of the signal-producing system can be located in the sold phase zone. After the solid phase has been adequately washed, a reagent required to detect the labelling can be released at a retarded rate in various embodiments in the heterogeneous immunoassay with detection in the bound phase. The following are possible examples:

The application of reagents by means of a stream of liquid arranged parallel to the main stream of liquid, but flowing more slowly and starting from the mobile phase reservoir and entering in front of the zone containing the labelled component. The parallel stream of liquid can be controlled by using an absorbent medium which chromatographs more slowly, for example a paper which chromatographs suitably slowly or a paper which is impregnated in places with "components temporarily blocking the way", such as, for example, polymers which impart a high viscosity on passing into solution (for example polyvinyl alcohols or dextrans).

After the solid phase has been adequately washed (=completion of chromatography), the application of reagents can be effected by pressing down an element which is a solid constituent of the test element. The "pressing down" can be effected mechanically or by removing distance pieces by the action of a stream of liquid. For example, the mechanical pressing down of an element containing the reagents can be effected by pressing down a flap or a piece of paper supported by distance pieces. The lowering of an element containing the reagents by the action of the stream of liquid can be effected, for example, by laminating over one another the solid phase, a water-soluble polymer and the reagent carrier (for example a suitably impregnated piece of paper).

A retarded introduction of reagents into the liquid stream can be effected using a microencapsulated reagent which only emerges from the encapsulation after the solid phase has been adequately washed, or by coating the reagent adhering in the matrix with components which dissolve slowly.

One possible means presented for the special case of enzyme labelling is as follows: when a peroxidase labelling is used, a glucose oxidase zone can be placed in front of the solid phase zone. Glucose and also the chromogen are then incorporated into the liquid stream, which can result in color formation behind the glucose oxidase. Appreciable color formation is only observed

if, at an appropriately high concentration of peroxidase, sufficient H_2O_2 is formed by the oxidase. This formation of the peroxide sets in slowly, reaches an optimum concentration and finally reaches a high concentration which results in inhibition of the enzyme and thus automatic cessation of the color formation. This coloration can be moderated if an H_2O_2-acceptor, for example a thioether as a mild reducing agent, or the enzyme catalase is incorporated in the oxidase zone or in front of the latter.

In this example a reagent for detecting the labelling is produced by a delay circuit, making use of an enzyme. The color formation in the solid phase zone only begins after this zone has been adequately washed free from nonspecifically bound labelling by the stream of liquid.

There are several possible means of preparing the solid phase zone. The components fixed there can be attached by chemical covalent bonds or adsorptively to an absorptive support which is a part of the test element. These components can also be attached to a dispersion of particles which remain fixed at the place of application after they have been applied to an absorbent support. For example, suspensions of cells carrying specific receptors on their surface, such as, for instance, Staphylococcus aureus Cowan I cells, or latex particles carrying combination partners of biological affinity attached to their surface, are suitable for being fixed in a paper matrix. The components of the test strip which are attached to pipettable supports and also the unattached components of the device can be dried onto the absorbent matrix of the element by air drying; freezedrying stages are not absolutely necessary.

A few test performance will be illustrated as examples of embodiments which can be regarded as independent of the labelling used. For the sake of simplicity, they are only described for the detection of a single analyte by means of the diagnostic device.

The following two embodiments, which conform to the principle of competitive immunoassay, will be described for the case where the analyte has only a single combination point of biological affinity or only one combination point of biological affinity out of several is utilized:

The solid phase combination partner is attached by covalent bonds or adsorptively to the supporting material of the solid phase functional region. The solution of analyte renders mobile a predetermined amount of labelled analyte contained in the diagnostic agent. The two components migrate into the functional sector containing the solid phase combination partner and compete for combination with the solid phase combination partner. If the proportion of analyte is high compared with the labelled analyte, little labelled analyte will be attached. If it is low, a great deal of labelled analyte will be attached.

The solid phase combination partner is housed as an unattached component in a functional region in front of the solid phase functional region. The oncoming front of solvent transports it into the solid phase functional region, where it becomes attached. This solid phase attachment is produced by combination systems of biological affinity which are independent of the combination system of the analyte. A combination partner which is conjugated with biotin attaches itself to avidin attached to the support. An immunoglobulin, such as IgG, as a combination partner, is fixed via its Fc component to support-attached protein A of S. aureus, or is attached by solid phase antibody of another species, non idiotypically directed to said immunoglobulin.

As previously described, the analyte and the labelled analyte compete, as constituents of the diagnostic agent, for the attachments to the solid phase combination partner during the processing period. This competition reaction takes place partly with the dissolved solid phase combination partner and partly with the solid phase combination partner which has already been attached to the solid phase.

If two combination points of differing specificity are present in an analyte, several embodiments, conforming to the principle of sandwich immunoassay, of the diagnostic agent are conceivable. Two of these will also be illustrated below:

If the solid phase combination partner is attached by covalent bonds or adsorptively to the supporting material of the solid phase functional region, the analyte forms, with the labelled combination partner, a binary complex which migrates together with the solvent into the solid phase functional region and reacts there with the solid phase combination partner, with the formation of a ternary complex, attached to the solid phase, which can be detected via the labelling of the first combination partner. The excess labelled combination partner is removed by the solvent into the subsequent functional region, the absorption zone.

If the solid phase combination partner is present in a non-attached form in the diagnostic agent and is rendered mobile by the solvent, the two reactants of the analyte of biological affinity are housed in the functional regions in such a way that the analyte reacts simultaneously or successively with both reactants and the resulting ternary complex then migrates into the solid phase functional region, where, as already described above, it becomes attached to the solid phase via a second system of biological affinity which is independent of that of the analyte.

In order to illustrate the embodiments descried above and further embodiments which conform to the immunometric test principle, the principle of indirect antibody detection or the ELA (enzyme-labelled-antigen) principle of immunoassay, FIGS. 1 and 2 illustrate in an exemplary manner the distribution of the components of the agent in the functional regions and, after the performance of the reaction, the composition of the solid phase complex, the amount of which is a measure of the concentration of analytes in the sample.

It has been found that a completely integrated test strip operating in accordance with the principle of heterogeneous immunoassay by means of solid phase detection is not only feasible in principle, but can, in addition, also be evaluated within a period of less than one hour, the quantification and the sensitivity of conventional RIAs or ELISAs being achieved. The detection of trace components in the range of 10^{-12} mol/liter has been made possible at reaction times of less than 30 minutes, at room temperature, the amounts of sample required being 10^{-16} mol, corresponding, for example, to approx. 1 pg. The arrangements described also enable tests of lower sensitivity requirements to be carried out, however. Standard curves over two to three decades were obtained when evaluation was carried out with the Sanoquell reflectometer (made by Quelle). The chromatography time for the test element, including complete color development, is not more than 16 minutes. Evaluation can also be carried out visually. With HCG as analyte, the start of the range of determination

in an example using a glucose oxidase attached to a solid phase and a peroxidase labelling was 0.3 ng/ml (corresponding to 3 U/liter).

In the example following, the application of the principle of the competitive double antibody test is presented as a concrete embodiment. In this test configuration, four components have to be reacted successively for the determination reaction and the separation stage, the reaction times and the concentrations of the reactant being critical values. The example is not to be regarded as limiting in any way, but merely serves to illustrate the subject of the invention further.

EXAMPLE

Completely integrated enzyme-immunochemical device for the detection of HCG by means of a built-in chromogen substrate system.

1.1. Reagents

1.1.1. HCG-peroxidase conjugate

HCG having a specific activity of approx. 3000 U/mg was obtained from Organon. Peroxidase from horseradish was obtained from Boehringer Mannheim (catalog no. 413,470). The hetero-bifunctional reagent N-γ-maleimidobutyryloxysuccinimide (GMBS) was obtained from Behring Diagnostics and was reacted with the HCG as described by Tranimori et al., 1983, in J.Imm. Meth. 62, 123-131. 2-iminothiolane hydrochloride (Sigma, catalog no. I 6256) was reacted with peroxidase as described by King et al., 1978, in Biochemistry 17, 1499-1506. A conjugate was prepared from the GMBS-HCG and the iminothiolane-peroxidase as described by Tanimori et al. The crude conjugate was purified by gel chromatography over Ultrogel ACA 44 (LKB). The fraction in which about 1-2 peroxidase molecules were coupled per HCG molecule was used for the test. The conjugate was diluted with Enzygnost IgE incubation medium made by Behringwerke, order no. OS D, designated briefly as incubation medium in the following text.

1.1.2. Antibodies

Antibodies against HCG were obtained by immunizing rabbits, and antibodies against rabbit-IgG were obtained by immunizing goats. The IgG fractions were isolated from serum by ammonium sulfate precipitation and anion exchange chromatography, and were purified further by immunadsorption. The methods used are described in the book "Immunologische Arbeitsmethoden" (Immunological working methods), Helmut Friemel, Editor, 1984, Gustav Fischer Verlag, Stuttgart. The anti-HCG antibody was finally diluted in the conjugate dilution buffer indicated above.

1.1.3. Glucose oxidase

Glucose oxidase from Aspergillus niger was obtained as a solution containing 300 U/mg (Serva, catalog No. 22,737). The glucose oxidase was finally diluted with incubation medium.

1.1.4. Glucose and Tetramethylbenzidine

α-D-glucose and tetramethylbenzidine hydrochloride were obtained from Serva, catalog no. 22,720 and 35,926, respectively.

1.2. Preparation of the Device

The sheet-like functional regions were prepared as follows:

The mobile phase application zone was prepared by cutting, to dimensions of 20×6 mm, a fabric sponge cloth made by Kalle; this is a synthetic sponge of regenerated cellulose which has been compressed in a dry state. It was impregnated with a solution of 50 mg of glucose and 0.75 mg of tetramethylbenzidine hydrochloride per ml of water, and was dried in a stream of air.

The conjugate, the anti-HCG antibody and glucose oxidase (5 μl of each at 25 μl/ml, 100 μl/ml and 0.1 mg/ml, respectively) were applied behind one another, at uniform distance, to a 45×5 mm piece of MN no. 1 paper (Macherey & Nagel), and were dried in the air.

A piece measuring 5×5 mm of Schleicher & Schull No. 597 paper was coated in a covalent manner with anti-rabbit IgG-antibody as the solid phase zone. This was effected by coupling the antibody with the paper, which had been activated with cyanogen bromide, as described by Clarke et al., 1979, Meth.Enzymology, volume 68, 441-442.

A 20×5 mm piece of Schleicher & Schüll No. 2668/8 paper was used as the absorption zone.

The four pieces of paper, with a 0.5-1 mm overlap behind one another, were fixed on a plastic ribbon by means of double-sided adhesive tape (Tesaband made by Beiersdorf), so that a test strip 5 mm wide was formed.

1.3. Performance of the Test

The test was carried out in each case by applying 200 μl of an HCG dilution in incubation medium to the fabric.

1.4 Results

The chromatographic development of the test element and the self-actuating color development were complete after 15 minutes at room temperature, and evaluation could be carried out either visually or by means of a reflectometer.

The following values were obtained when evaluating the solid phase zone (No. 597 paper) with the Sanoquell blood glucose evaluation apparatus made by Quelle:

HCG concentration (U/liter)	Measured values (mg of glucose per dl of blood)
0.3	107
3	117
30	95
300	70
3000	0

The following values were obtained with the same test strips using the Rapimat urine test strip evaluation apparatus made by Behringwerke:

HCG concentration (U/liter)	Measured values (BIT)
0.3	76
3	76
30	94
300	119

-continued

HCG concentration (U/liter)	Measured values (BIT)
3000	135

The test strip assembly shown here can also be achieved if the glucose oxidase and the anti-HCG antibody are located in the same zone. The test strip, which is correspondingly shorter, then renders the result after approx. 10 minutes.

We claim:

1. An analytical device for the detection or determination of a component in a fluid wherein said component is an analyte with bioaffinity binding properties, comprising a layer of a plurality of substantially planar zones adjacent one another and in absorbent contact with one another, said layer including:

a mobile phase application zone (MPAZ), an intermediate zone (IZ) and an adsorption zone (AZ), liquid being capable of moving by adsorption from said MPAZ through said IZ to said AZ, and wherein said IZ further comprises a solid phase zone (SPZ) having at least one unlabelled reactant, capable of interactions of biological affinity with at least one analyte;

at least one unattached, labelled reactant (conjugate), capable of interactions of biological affinity with said at least one analyte, disposed in an area between the MPAZ and the SPZ; and

an analyte application zone disposed at said MPAZ or in between said MPAZ and said AZ, wherein after application of said at least one analyte, said at least one analyte is reacted with said reactants in said layer and is detected in said layer.

2. A device as claimed in claim 1, wherein the MPAZ has the function of a volume metering element and releases to the subsequent zones at least sufficient liquid for the liquid, controlled by capillary forces, to reach the end of the AZ.

3. A device as claimed in claim 1, wherein the MPAZ is a plastic sponge or a particulate layer which is composed of hydrophilic polymers and which is capable of containing chemicals, buffer substances or other substances required for certain tests.

4. A device as claimed in claim 1, wherein the analyte application zone retains blood cells.

5. A device as claimed in claim 1, wherein all or some of the reagents required for the detection of the labelling are present in one or more of substantially planar zones of the device.

6. A device as claimed in claim 1, wherein said at least one unlabelled reactant is fixed to said SPZ by means of covalent bonds.

7. A device as claimed in claim 1, wherein said at least one unlabelled reactant is fixed to said SPZ by means of absorption.

8. A device as claimed in claim 1, wherein said at least one unlabelled reactant is fixed to said SPZ by means of an interaction of biological affinity.

9. A device as claimed in claim 1, further including a plurality of solid phase zones (SPZs) for the detection of a plurality of analytes, said analytes including at least one attachment point of biological affinity, each of said SPZs being adjacent one another in said layer and each of said SPZs including said unlabelled reactants fixed thereto, said unlabelled reactants of each SPZ being specific for a specific analyte to be detected in each of said SPZs.

10. A device as claimed in claim 1, wherein said layer includes a chromotographing section in at least a portion of said substantially planar zones, and further including a sample application zone laminated onto at least a portion of said chromatographing section and in adsorptive contact therewith.

11. A device as claimed in claim 1, wherein said layer includes a chromotographing section in at least a portion of said substantially planar zones, and further including a reagent zone laminated onto at least a portion of said chromotographing section and in adsorptive contact therewith, wherein at least some of the reagents required for the detection of the labelling are present in said reagent zone.

12. A process for the detection or determination of a component in a fluid wherein said component is an analyte with bioaffinity binding properties by rehydrating or solvating reactants and reagents by the fluid containing the analyte or by an additional fluid, said reactants and reagents being present in a dehydrated state in an analytical device for the detection or determination of a component in a fluid wherein said component is an analyte with bioaffinity binding properties, comprising a layer of a plurality of substantially planar zones adjacent one another and in absorbent contact with one another, aid layer including:

a mobile phase application zone (MPAZ), an intermediate zone (IZ) and an adsorption zone (AZ), liquid being capable of moving by adsorption from said MPAZ through said IZ to said AZ, and wherein said IZ further comprises a solid phase zone (SPZ) having at least one unlabelled reactant, capable of interactions of biological affinity with at least one analyte;

at least one unattached, labelled reactant (conjugate), capable of interactions of biological affinity with said at least one analyte, disposed in an area between the MPAZ and the SPZ; and

an analyte application zone disposed at said MPA or in between said MPAZ and said AZ,

said process comprising:

applying a sample to said analyte application zone, reacting the at least one analyte in the sample in said layer and detecting said at least one analyte in said layer.

13. The process as claimed in claim 12, wherein, after the liquid sample containing the analyte has been fed to the MPAZ or after the sample has been fed to a sample application zone and a mobile phase has been fed to the MPAZ, the liquid reaches the end of the AZ, under the control of capillary forces, and reactions between reactants contained in the device and the analyte are thereby set in operation, and, after the labelled reactants which are not attached to the solid phase have been removed chromatographically, the amount of the labelling in the solid phase zone, which is a measure of the analyte concentration in the sample, is determined.

14. The process as claimed in claim 12, wherein the reactions taking place in the device are based on the principals of at least one of immunological detection reactions, competitive immunometric or sandwich immunoassay, indirect antibody detection by means of a labelled antibody and antibody detection by means of a labelled antigen.

15. The process as claimed in claim 12, wherein said detecting includes using a fluorophor as a labelling

agent which is detected or measured directly or is detected or measured after the addition of a reagent present in the device, or a fluorophor which is detected or measured directly or after the addition of a further reagent is formed from the labelling agent by the addition of a reagent present in the device.

16. The process as in claim 12, wherein said detecting includes using a compound which can be excited to give chemiluminescence as a labelling agent, the chemiluminescence being detectable or measurable after the addition of a reagent present in the device.

17. The process as claimed in claim 12, wherein said detecting includes using an enzyme as a labelling agent, the activity of which is determined with the aid of a reagent present in the device.

18. An analytical device for the detection or determination of a component in a fluid wherein said component is an analyte with bioaffinity binding properties, comprising a layer of a plurality of sheet-like zones adjacent one another and in absorbant contact with one another, said layer including:

a mobile phase application zone (MPAZ), an intermediate zone (IZ) and an adsorption zone (AZ), liquid being capable of moving by adsorption from said MPAZ through said IZ to said AZ, and wherein said IZ further comprises a solid phase zone (SPZ) capable of having at least one unlabelled reactant fixed thereto which is capable of interactions of bioaffinity with at least one analyte, during analysis said at least one unlabelled reactant being fixed to at least one second reactant which is fixed to said solid phase zone;

at least one unattached labelled reactant (conjugate), capable of interactions of biological affinity with said at least one analyte, disposed in an area between said MPAZ and said SPZ; and

an analyte application zone disposed at said MPAZ or in between said MPAZ and said AZ, wherein after application of said at least one analyte, said at least one analyte is reacted with said reactants in said layer and is detected in said layer.

19. A device as claimed in claim 18, wherein said at least one second reactant is fixed to said SPZ by means of covalent bonds.

20. A device as claimed in claim 18, wherein said at least one second reactant is fixed to said SPZ by means of adsorption.

21. A device as claimed in claim 18, wherein said at least one second reactant is fixed to said SPZ by means of an interaction of biological affinity.

22. A device as claimed in claim 18, further including a plurality of solid phase zones (SPZs) for the detection of a plurality of analytes, said analytes including at least one attachment point of biological affinity, each of said SPZs being adjacent one another in said layer and each of said SPZs including said unlabelled reactants fixed thereto, said unlabelled reactants of each SPZ being specific for a specific analyte to be detected in each of said SPZs.

23. A device as claimed in claim 18, wherein the MPAZ has the function of a volume metering element and releases to the subsequent zones at least sufficient liquid for the liquid, controlled by capillary forces, to reach the end of the AZ.

24. A device as claimed in claim 18, wherein the MPAZ is a plastic sponge or a particulate layer which is composed of hydrophilic polymers and which is capable of containing chemicals, buffer substances or other substances required for certain tests.

25. A device as claimed in claim 18, wherein the analyte application zone retains blood cells.

26. A device as claimed in claim 18, wherein said layer includes a chromotographing section in at least a portion of said substantially planar zones; and further including a sample application zone laminated onto at least a portion of said chromatographing section and in adsorptive contact therewith.

27. A device as claimed in claim 18, wherein all or some of the reagents required for the detection of the labelling are present in one or more of the substantially planar zones of the device.

28. A device as claimed in claim 18, wherein said layer includes a chromotographing section in at least a portion of said substantially planar zones, and further including a reagent zone laminated onto at least a portion of said chromotographing section and in adsorptive contact therewith, wherein at least some of the reagents required for the detection of the labelling are present in said reagent zone.

29. A process for the detection or determination of a component in a fluid as an analyte with bioaffinity binding properties by rehydrating or solvating reactants and reagents by the fluid containing the analyte or by an additional fluid, said reactants and reagents being present in a dehydrated state in an analytical device for the detection or determination of the analyte, said device including a layer of a plurality of substantially planar zones adjacent one another and in absorbent contact with one another, said layer including:

a mobile phase application zone (MPAZ), an intermediate zone (IZ) and an adsorption zone (AZ), liquid being capable of moving by adsorption from said MPAZ through said IZ to said AZ;

a solid phase zone (SPZ) in said IZ capable of having at least one unlabelled reactant fixed thereto which is capable of interactions of bioaffinity with at least one analyte, during analysis said at least one unlabelled reactant being fixed to at least one second reactant which is fixed to said solid phase zone;

at least one unattached labelled reactant (conjugate), capable of interactions of biological affinity with said at least one analyte, disposed in a zone between the MPAZ and the SPZ; and

an analyte application zone disposed at said MPAZ or in between said MPAZ and said AZ;

said process comprising:

applying a sample to said analyte application zone, reacting the at least one analyte in the sample in said layer and detecting said at least one analyte in said layer.

30. The process as claimed in claim 29, wherein, after the liquid sample containing the analyte has been fed to the MPAZ or after the sample has been fed to a sample application zone and a mobile phase has been fed to the MPAZ, the liquid reaches the end of the AZ, under the control of capillary forces, and reactions between reactants contained in the device and the analyte are thereby set in operation, and, after the labelled reactants which are not attached to the solid phase have been removed chromatographically, the amount of the labelling in the solid phase zone, which is a measure of the analyte concentration in the sample, is determined.

31. The process as claimed in claim 29, wherein the reactions taking place in the device are based on the principals of at least one of immunological detection

reactions, competitive immunometric or sandwich immunoassay, indirect antibody detection by means of a labelled antibody and antibody detection by means of a labelled antigen.

32. The process as claimed in claim 29, wherein said detecting includes using a fluorophor as a labelling agent which is detected or measured directly or is detected or measured after the addition of a reagent present in the device, or a fluorophor which is detected or measured directly or after the addition of a further reagent is formed from the labelling agent by the addition of a reagent present in the device.

33. The process as in claim 29, wherein said detecting includes using a compound which can be excited to give chemiluminescence as a labelling agent, the chemiluminescence being detectable or measurable after the addition of a reagent present in the device.

34. The process as claimed in claim 29, wherein said detecting includes using an enzyme as a labelling agent, the activity of which is determined with the aid of a reagent present in the device.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,861,711

DATED : August 29, 1989

INVENTOR(S) : Heinz-Jürgen Friesen et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Claim 12, column 10, line 28, "aid" should read --said --.

Claim 18, column 11, line 19, "sheet-like" should read substantially planar --.

Signed and Sealed this

Twenty-third Day of October, 1990

Attest:

HARRY F. MANBECK, JR.

Attesting Officer

Commissioner of Patents and Trademarks